

SMART PRACTICES FOR PRODUCTION OF CHALLENGE AGENTS

Human Infection Studies (also known as Controlled Human Infection Studies/Models or Challenge studies) are defined as biomedical research whereby volunteers are intentionally exposed to viable infectious agents (challenge agents) under carefully controlled conditions to further understanding of different diseases, infectious processes and human immune responses.

The Smart Practices are a summary of the [“Considerations on the principles of development and manufacturing qualities of challenge agents for use in human infection models”](#)¹, a document developed by a consortium of international experts to discuss fundamental principles of selection, characterisation, manufacture, quality control and storage of challenge agents for international reference. They do not form regulatory guidelines or guidance but reflect the consortium’s recommendations in the absence of clear regulatory guidance.

The core principles summarised in this document encompass:

- Challenge agent characteristics and clinical study design
- Engagement with regulatory bodies
- Selecting and characterising challenge agent, with reference to desired study objectives
- Production considerations, including; equipment/reagents, personnel, facilities, documentation, environmental monitoring, and shipping/transportation.
- QC and testing (identity, purity, potency and stability)
- Storage and monitoring long-term challenge agent stability
- Keeping a challenge agent dossier

The principles aim to encompass viral, bacterial and parasitic challenge agents, but cannot provide specific details for every infectious agent. Additionally, while genetic modification of challenge agents and safety reporting are referred to, they are beyond the scope of this document. The principles apply to challenge agents made to GMP guidelines and those made using GMP-like procedures and facilities (there are published helpful discussions of the regulatory requirements for challenge agents²). A list of related relevant guidelines, including ICH guidelines, can be found on page 7 of this document.

A list of case studies can be found in Appendix 1 on page 9.

Section 1: PLANNING

The challenge agent characteristics will influence the design of the clinical study and manufacturing requirements. Considerations:

- Dose escalation study (starting dose may be informed by natural infective dose).
- Administration route (oral, intravenous, insect bite, transdermal, inhalation, intranasal, topical) will affect quality considerations and level of purity required. The inclusion of vectors necessitates additional considerations of local vector suitability, associated contaminants and impacts on the manufacturing process.
- Risk assessment (will inform containment levels, mitigation strategies and study design).
- Setting identity, potency and purity specification limits.

Section 2: ENGAGEMENT WITH REGULATORY BODIES

Early engagement with regulatory bodies is recommended. Considerations:

- Relevant agencies overseeing health and safety in the work-place laws.
- National scientific advisory groups for handling infectious agents.
- International/National/local/Institutional Biosafety Committees
- National Agency(ies) regulating medicines and medical devices (some countries regulate challenge agents as Investigative Medicinal Products, others do not; either way it is recommended to engage).
- Public health protection (national and local) to manage the risk of environmental transmission (infectious agents may have the potential to be transmitted to others such as household members, study staff, the community, and to the environment). Risk assessments should be conducted and recorded for all manipulations with the challenge agent.
- GMO challenge agents to be discussed and registered with the relevant national regulatory agency and regulations for deliberate release of a GMO should be followed (cost and timeline impacts should be considered too).
- Engagement with regulatory bodies will also be required to import challenge agents for clinical use.

Section 3: CHALLENGE AGENT SELECTION AND CHARACTERISATION

Selection:

The challenge agent may be wild-type, adapted and/or attenuated from wild-type to reduce pathogenicity, or genetically modified. Considerations:

- Representation of current strains causing natural infection/disease and relevance of model
- Meeting requirements for virulence/attack rate
- Model that is fit for purpose (e.g. if testing a vaccine, is the relevant antigen present)
- Manageable symptom profile, sensitivity to treatment, and/or self-limiting infection in target demographic
- Risk/benefit profile of therapies
- Clearance test(s) i.e. the availability of rapid diagnostic tests or other tests to show that treatment has been successful and/or the infection has resolved throughout the study
- Impacts of adaptations, attenuation and genetic modification on challenge agent characteristics
- Impacts on manufacturing process and biosafety containment
- Environmental impacts (accidental release of non-endemic/genetically modified strains or hosts)
- Risk and safety profiles of any required vectors

The source of the challenge agent will affect aspects of manufacturing processes and risk assessments. Considerations:

- Purity (risk and nature of contaminants in the isolate preparation)
- Compliance with the [Nagoya Protocol](#) (particularly if the challenge agent is isolated from another country)
- Provenance of the isolate (and clinical profile)

Section 4: PRODUCTION

If producing a challenge agent outside of GMP conditions and facilities, the key considerations are:

4.1 Equipment and Reagents

All equipment should have unique identifiers, be in service/calibrated (with records available), be decontaminated before use, and dedicated to challenge agent preparation to avoid cross contamination.

- It is recommended that any ancillary equipment e.g. cryovials, conical tubes etc. used should be sterile, from an unopened package.
- Ancillary equipment can be placed under UV in a safety cabinet 24 hours before being used.

Reagents should be purchased ahead of time and any formulations prepared/reagents aliquoted a few days prior to use.

4.2 Procedures

Considerable planning is required when producing a challenge agent. This includes but is not limited to:

- Resourcing; trained personnel are required to handle the challenge agent when being propagated for seed and master bank stocks under appropriate room conditions (to prevent contamination and protect personnel from infection). Two individuals (1 for conducting the procedures and 1 for verification) are required on any day.
- Testing of the challenge agent may be performed under standard laboratory conditions i.e. there are no requirements for a clean room.
- Facilities; ideally the laboratory used for propagating the challenge agent must solely be used for this purpose and decontaminated prior to use.
- Forms for batch and aliquot records as well as challenge agent propagation can be prepared ahead of time in addition to laboratory notebooks.

Labelling: templates of labels can also be prepared ahead of time leaving the dose/concentration and dates blank. Note: ensure if vials are being frozen suitable labels are purchased. See Section 5.2 ('Final product considerations') for labelling requirements.

4.3 Documentation

a) Challenge Agent source and characterisation

Full provenance of the starting material must be known e.g. if the source is from a human donor then the medical history and outcome of the infection for the individual (please note the individual should not be identifiable) must be recorded and kept.

All identity, purity and potency testing and any subsequent stability testing information need to be kept as part of the dossier for the challenge agent.

b) Production of Challenge Agent

Details of any reagents prepared or aliquoted during the production of the challenge agent stock should be provided and follow a Standard Operating Procedure (SOP). Details that need to be

recorded include identification of reagent(s) being mixed/aliquoted, batch and/or lot number, expiry date, concentration/dose (if applicable), date of preparation, volume used, and the initials of the individual who made the preparation. Preprepared aliquots may be given identifying numbers that can also be recorded.

All manipulations including the initial propagation of the challenge agent performed need to be recorded, dated and signed with details of the following:

- Equipment – unique identifier/serial number
- Ancillary Equipment e.g. lot numbers and expiry date
- Reagent details (as specified above)

Risk Assessment should be prepared for the challenge agent covering receipt/storage, all manipulations e.g. propagation, dilution etc. and disposal (alongside assessments required by national governing bodies covering substances hazardous to health).

c) Temperature and Environmental Monitoring

Temperature records for any freezers, water baths, incubators, thermologgers (e.g. if used during transient storage) etc. should be recorded and kept. For those with continuous recording of data these should be available. Temperature loggers or thermometer used in the manufacturing or storage processes must be calibrated. All results of any environmental monitoring should be kept.

d) Shipping and Receipt





Chain of custody documentation should accompany any challenge agent being transported and should cover:

- Identification of Challenge Agent and potency
- Number of vials sent
- Batch number
- Storage Conditions e.g. on ice/room temperature and period of use
- Date and Time sent
- Name of shipper/courier
- Name, address and contact number of Sponsor, manufacturing organisation, CI/PI of clinical trial (and trial reference number if available)
- The wording “For clinical trial use only”
- Directions for use

Stability tests under different transportation/shipping and packaging conditions should be used to validate the suitability of packaging and packaging-material to preserve potency, purity and biological activity. A calibrated temperature logger can be included in the shipment to ensure the correct temperature is maintained.

The labelling information should comply with any relevant national laws or requirements and the labelling operation performed at the manufacturing site. Secondary packaging should be validated, for example with respect to temperature control.

Example of a shipping label:

Name:	(Enter name of challenge agent)	
Trial No:	(Enter trial reference)	
Identifier(s):	(Enter full challenge agent ID e.g. strain)	
Concentration:	(Enter concentration and volume)	
Quantity of units:	(Enter number of vials)	
Route of administration:	(Enter ROA)	
LOT:	(Enter batch number)	
	(Enter temperature range)	
	dd-MMM-yy	 dd-MMM-yy
Sponsor:	(Enter sponsor details)	
For Clinical Trial Use Only; (Add any other relevant restrictions)		
Version no. etc.		

The receiver should check the package once they receive it and confirm with the shipper that (1) they have received the correct shipment with the same number of vials sent and that (2) the vials have not been compromised in anyway e.g. there is no leakage or evidence of tampering.

Any temperature loggers included in the shipment should have the data downloaded and checked to ensure that the temperature did not exceed the predetermined set limits. Any deviation outside these limits will need a full risk assessment on potential quality and strategies to assess possible impacts.

The receiver should perform quality control testing and stability (identity, purity, and potency) checks on at least two independent vials after transportation and before the challenge agent is put into use as stipulated in the 'QC and Testing' and 'Storage and Stability' sections.

Section 5: QC AND TESTING

5.1 Characterisation

Challenge agent characterisation encompasses the Critical Quality Attributes: “a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality” and confirm the challenge agent meets the specifications set out in the planning phase.

Assays for characterisation and quality control testing should be fit-for-purpose, qualified assays (e.g. *in vitro* potency assays and *in vivo* data) for characterisation.

Identity:

- Identity: genotype, serotype, subtype. Methods: whole genome sequencing (coverage and length of read), MALDI-TOF, other appropriate assays. Include integrity of genetic modification if applicable.
- Impurities and degradation products: non-viable agent, toxins.
- Sensitivity to treatment(s).

- Physical characteristics: size, sensitivity to pH, temperature, UV, chemicals.
- Excipients: assessment/removal of carry over reagents from manufacture process. Diluents should be justified and carefully selected.

Purity:

- Microbial limits/Adventitious agent testing: challenge agents should be free from other infectious agents of concern to humans (animal/human viruses, bacteria, fungi, endotoxin, mycoplasma) and use of animal and/or human derived products in manufacturing should be avoided as much as possible. Whole genome sequencing, with the appropriate controls, could be considered as a route of testing.
- Refer to national guidelines/guidance detailing recommendations on what chemistry, manufacturing, and controls (CMC) information should be included regarding the reporting, identification, and qualification of impurities in drug products.

Potency:

- Potency/biological activity (e.g. viability, infectivity *in vitro/in vivo*)

Stability (identity, purity and potency over time):

- Stability during and following manufacture, transport and storage: reference material(s) if applicable, period/intervals of testing linked to stability characteristics.

5.2 Final product considerations

- A specification for the challenge agent should be defined.
- Challenge agent release: Label the vials with ID of challenge agent, volume and quantity/concentration of organisms, date prepared, date of expiry (usually an arbitrary date), storage conditions and initials of the operator (labels should comply with PIC/S Annex 13, see reference documents).
- Consistency of fill and potency in each of the containers or vial fills.
- Dosage form: fresh, lyophilised or cryopreserved (consider impact on potency and utility for route of administration).
- Single dose or multi-dose vials (if multi-dose consider suitability of site for inoculum preparation).
- Container closure: impact on integrity and target shelf-life.
- Container labelling and secondary packaging.
- Retention samples are required for critical raw materials and final product.

Section 6: STORAGE AND STABILITY

- Real-time stability studies during manufacturing process, storage over time, and freeze/thawing steps.
- Stability tests upon receipt of challenge agents from another laboratory
- In-use stability studies: potency at time of inoculum preparation and after administration (can include back-titration of inoculum and retention samples).

There are potentially two types of stability to consider:

1) Phenotypic and genetic stability of the agent due to possible mutations during the process: For some infectious agents, number of passages can have an impact on genome stability. Viruses and bacteria can be subject to mutations in their genome during the manufacturing process with an impact on challenge agent stability. Pathogenicity, viability, motility and morphology should be also considered.

2) Stability profile in terms of degradation, viability and potency: Stability studies should cover real-time stability to determine the shelf-life and also in-use stability to determine the instructions at the clinical trial site (for example, for containers with more than one dose, the following information should be provided: storage duration and conditions once the container is open).

Stability studies can include annual viability/titre determination (to ensure there is no loss after time) and microbial contamination testing.

Section 7: CHALLENGE AGENT DOSSIER

This should include:

- Protocol for clinical use at trial site
- Contraindications
- Warnings and precautions
- Adverse reactions
- Medicinal/drug interactions
- Use in specific populations
- Waste management.

Section 8: RELATED GUIDELINES

The following are provided for reference. They may assist establishing relevant procedures and avoid missing important items related to the infectious agent manufacture.

Current versions of the ICH guidelines can be found on the ICH website (<https://www.ich.org/>):

ICHQ5a: Viral safety evaluation of Biotechnology products derived from cell lines of human or animal origin

ICHQ5c: Quality of biotechnological products: Stability testing of biotechnological/biological products

ICHQ5d: Derivation and characterisation of cell substrates used for production of biotechnological/biological products

ICHQ 6b: Specifications: test procedures and acceptance criteria for biotechnological/biological products

ICHQ 7: Good manufacturing practice

ICHQ 8: Pharmaceutical development

ICHQ 9: Quality risk management

ICHQ 10: Pharmaceutical quality system

ICHQ 11: Development and manufacture of drug substances

Current versions of the ISO guidelines can be found on the ISO website (<https://www.iso.org/home.html>):
ISO 9001:2015 Quality management systems - requirements

Current versions of the PIC/S guidelines can be found on the PIC/S website (<https://picscheme.org/en/picscheme>):
PE 009-16 (Annexes) Guide to good manufacturing practice for medicinal products annexes

References

1. Balasingam S, Meillon S, Chui C, Mann A, La C, Weller CL, et al. Human infection studies: Key considerations for challenge agent development and production. Vol. 7, Wellcome Open Research. F1000 Research Ltd; 2022.
2. Bekerredjian-Ding I, Van Molle W, Baay M, Neels P; PEI speakers and session chairs. Human challenge trial workshop: Focus on quality requirements for challenge agents, Langen, Germany, October 22, 2019. *Biologicals*. 2020 Jul;66:53-61. doi: 10.1016/j.biologicals.2020.04.005.

Appendix 1. Case studies for infectious agent manufacture (human use)

Pathogen	Title	Reference
<i>Influenza</i>	Continuous cell lines as a production system for influenza vaccines	Genzel Y, Reichl U. Continuous Cell Lines as a Production System for Influenza Vaccines. Expert Review of Vaccines. 2009 Dec 1;8(12):1681-92.doi: https://doi.org/10.1586/erv.09.128
<i>Human orthopneumovirus</i> (Respiratory syncytial virus)	Viral Load Drives Disease in Humans Experimentally Infected with Respiratory Syncytial Virus	DeVincenzo JP, Wilkinson T, Vaishnav A, Cehelsky J, Meyers R, Nochur SV, et al. Viral Load Drives Disease in Humans Experimentally Infected with Respiratory Syncytial Virus. American Journal of Respiratory and Critical Care Medicine. 2010 Nov 15;182(10):1305–14. doi: https://doi.org/10.1164/rccm.201002-0221OC
<i>Norovirus</i>	Predicting Susceptibility to Norovirus GII.4 by Use of a Challenge Model Involving Humans	Frenck R, Bernstein DI, Xia M, Huang P, Zhong W, Parker S, et al. Predicting Susceptibility to Norovirus GII.4 by Use of a Challenge Model Involving Humans. Journal of Infectious Diseases. 2012 Aug 20;206(9):1386–93. doi: https://doi.org/10.1093/infdis/jis514
<i>Rhinovirus</i>	A Tool for Investigating Asthma and COPD Exacerbations: A Newly Manufactured and Well Characterised GMP Wild-Type Human Rhinovirus for Use in the Human Viral Challenge Model	Fullen D, Murray BD, Mori J, Catchpole A, Borley DW, Murray E, et al. A Tool for Investigating Asthma and COPD Exacerbations: A Newly Manufactured and Well Characterised GMP Wild-Type Human Rhinovirus for Use in the Human Viral Challenge Model. PLoS One. 2016 Dec 9;11(12):e0166113–3. doi: https://doi.org/10.1371/journal.pone.0166113
<i>Betacoronavirus</i> (SARS-CoV-2)	Safety, tolerability and viral kinetics during SARS-CoV-2 human challenge in young adults	Killingley B, Mann AJ, Kalinova M, Boyers A, Goonawardane N, Zhou J, et al. Safety, tolerability and viral kinetics during SARS-CoV-2 human challenge in young adults. Nature Medicine. 2022 Mar 31;28(5):1031–41. doi: https://doi.org/10.1038/s41591-022-01780-9
<i>Shigella</i>	Consensus Report on <i>Shigella</i> Controlled Human Infection Model: Conduct of Studies	Talaat KR, Bourgeois AL, Frenck RW, Chen WH, MacLennan CA, Riddle MS, et al. Consensus Report on Shigella Controlled Human Infection Model: Conduct of Studies. Clinical Infectious Diseases. 2019 Dec 9;69(Supplement_8):S580–90.doi: https://doi.org/10.1093/cid/ciz892
<i>Salmonella</i> Typhi	An Outpatient, Ambulant-Design, Controlled Human Infection Model Using Escalating Doses of <i>Salmonella</i> Typhi Challenge Delivered in Sodium Bicarbonate Solution	Waddington CS, Darton TC, Jones C, Haworth K, Peters A, John T, et al. An Outpatient, Ambulant-Design, Controlled Human Infection Model Using Escalating Doses of Salmonella Typhi Challenge Delivered in Sodium Bicarbonate Solution. Clinical Infectious Diseases. 2014 Feb 10;58(9):1230–40. doi: https://doi.org/10.1093/cid/ciu078
<i>Salmonella</i> Paratyphi	Evaluation of the Clinical and Microbiological Response to <i>Salmonella</i> Paratyphi A Infection in the First Paratyphoid Human Challenge Model	Dobinson HC, Gibani MM, Jones C, Thomaides-Brears H, Merryyn Voysey, Darton TC, et al. Evaluation of the Clinical and Microbiological Response to Salmonella Paratyphi A Infection in the First Paratyphoid Human Challenge Model. 2017 Apr 15;64(8):1066–73. doi: https://doi.org/10.1093/cid/cix042

<i>Campylobacter</i>	Experimental <i>Campylobacter jejuni</i> infection in humans	Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ. Experimental <i>Campylobacter jejuni</i> Infection in Humans. The Journal of Infectious Diseases. 1988 Mar 1;157(3):472–9. doi: https://doi.org/10.1093/infdis/157.3.472
<i>Streptococcus pyogenes</i>	Controlled human infection for vaccination against <i>Streptococcus pyogenes</i> (CHIVAS): Establishing a group A <i>Streptococcus</i> pharyngitis human infection study	Osoewicki J, Azzopardi KI, Baker C, Waddington CS, Pandey M, Schuster T, et al. Controlled Human Infection for Vaccination Against <i>Streptococcus Pyogenes</i> (CHIVAS): Establishing a Group A <i>Streptococcus</i> Pharyngitis Human Infection Study. Vaccine. 2019 Jun;37(26):3485–94. doi.org/10.1016/j.vaccine.2019.03.059
<i>Bordetella pertussis</i>	Investigating <i>Bordetella pertussis</i> colonisation and immunity: protocol for an inpatient controlled human infection mode	De Graaf H, Gbesemete D, Gorringe AR, Diavatopoulos DA, Kester KE, Faust SN, et al. Investigating <i>Bordetella pertussis</i> colonisation and immunity: protocol for an inpatient controlled human infection model. BMJ open. 2017 Oct 1;7(10):e018594. doi: http://dx.doi.org/10.1136/bmjopen-2017-018594
<i>Neisseria</i>	Nasopharyngeal colonization by <i>Neisseria lactamica</i> and induction of protective immunity against <i>Neisseria meningitidis</i>	Evans CM, Pratt CB, Matheson M, Vaughan TE, Findlow J, Borrow R, et al. Nasopharyngeal Colonization by <i>Neisseria lactamica</i> and Induction of Protective Immunity against <i>Neisseria meningitidis</i> . Clinical Infectious Diseases. 2011 Jan 1;52(1):70–7. doi: https://doi.org/10.1093/cid/ciq065
<i>Neisseria</i>	Protocol for a controlled human infection with genetically modified <i>Neisseria lactamica</i> expressing the meningococcal vaccine antigen NadA: a potent new technique for experimental medicine	Gbesemete D, Laver JR, De Graaf H, Ibrahim M, Vaughan A, Faust S, et al. Protocol for a controlled human infection with genetically modified <i>Neisseria lactamica</i> expressing the meningococcal vaccine antigen NadA: a potent new technique for experimental medicine. BMJ Open. 2019 Apr;9(4):e026544. doi: http://dx.doi.org/10.1136/bmjopen-2018-026544
<i>Vibrio cholerae</i> (Cholera)	Response of Man to Infection with <i>Vibrio Cholerae</i> . I. Clinical, Serologic, and Bacteriologic Responses to a Known Inoculum	Cash RA, Music SI, Libonati JP, Snyder MJ, Wenzel RP, Hornick RB. Response of Man to Infection with <i>Vibrio Cholerae</i> . I. Clinical, Serologic, and Bacteriologic Responses to a Known Inoculum. Journal of Infectious Diseases. 1974 Jan 1;129(1):45–52. doi: https://doi.org/10.1093/infdis/129.1.45
<i>Plasmodium</i> (Malaria): Blood-stage	Controlled human malaria infection with a clone of <i>Plasmodium vivax</i> with high-quality genome assembly	Minassian AM, Themistocleous Y, Silk SE, Barrett JR, Kemp A, Quinkert D, et al. Controlled Human Malaria Infection with a Clone of <i>Plasmodium Vivax</i> with High-quality Genome Assembly. JCI Insight. 2021 Dec 8;6(23).doi: https://doi.org/10.1172/jci.insight.152465 .
<i>Plasmodium</i> (Malaria): Sporozoite	Infectivity of cultured <i>Plasmodium falciparum</i> gametocytes to mosquitoes	Ponnudurai T, Lensen AH, Van Gemert GJ, Bensink MP, Bolmer M, Meuwissen JT. Infectivity of Cultured <i>Plasmodium falciparum</i> Gametocytes to Mosquitoes. Parasitology. 1989 Apr;98(2):165–73. Doi: https://doi.org/10.1017/S0031182000062065
<i>Schistosoma mansoni</i>	Establishing the Production of Male <i>Schistosoma mansoni</i> Cercariae for a Controlled Human Infection Model	Janse JJ, Langenberg C, Janneke Kos-van Oosterhoud, Arifa Ozir-Fazalalikh, Eric, Beatrice, et al. Establishing the Production of Male <i>Schistosoma mansoni</i> Cercariae for a Controlled Human Infection Model. The Journal of Infectious Diseases. 2018 Aug 24;218(7):1142–6. doi: https://doi.org/10.1093/infdis/jiy275

<i>Necator americanus</i> (Hookworm)	Controlled Human Hookworm Infection: Accelerating Human Hookworm Vaccine Development	Diemert D, Campbell D, Brelsford J, Leasure C, Li G, Peng J, et al. Controlled Human Hookworm Infection: Accelerating Human Hookworm Vaccine Development. Open Forum Infectious Diseases. 2018 Apr 19; 5(5): ofy083. doi: https://doi.org/10.1093/ofid/ofy083
<i>Dengue virus</i>	Attenuation and immunogenicity in humans of a live dengue virus type-4 vaccine candidate with a 30 nucleotide deletion in its 3'-untranslated region	Durbin AP, Reynolds MJ, Chanock RM, Whitehead SS, Murphy BR, Elkins WR, et al. Attenuation and Immunogenicity in Humans of a Live Dengue Virus Type-4 Vaccine Candidate with a 30 Nucleotide Deletion in its 3'-Untranslated Region. The American Journal of Tropical Medicine and Hygiene. 2001 Nov 1;65(5):405–13.doi: https://doi.org/10.4269/ajtmh.2001.65.405
<i>Leishmania</i>	Characterization of a new <i>Leishmania major</i> strain for use in a controlled human infection model	Ashwin H, Sadlova J, Vojtkova B, Becvar T, Lypaczewski P, Schwartz E, et al. Characterization of a New Leishmania Major Strain for Use in a Controlled Human Infection Model. Nature Communications. 2021 Jan 11;12(1):215. doi: https://doi.org/10.1038/s41467-020-20569-3
<i>Streptococcus pneumoniae</i>	Human Nasal Challenge with <i>Streptococcus pneumoniae</i> Is Immunising in the Absence of Carriage	Wright AKA, Ferreira DM, Gritzfeld JF, Wright AD, Armitage K, Jambo KC, et al. Human Nasal Challenge with Streptococcus pneumoniae Is Immunising in the Absence of Carriage. PLoS Pathogens. 2012 Apr 5;8(4):e1002622. doi: https://doi.org/10.1371/journal.ppat.1002622