

# **How To Challenge – Current Design, Participant and Microbiological Considerations of Human Volunteer Challenge Studies.**

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## SUMMARY

Human volunteer challenge studies, also known as deliberate, controlled or experimental infection studies, have provided vital insights into the aetiology of human disease, mechanisms of pathogenesis, treatment and vaccination for at least 300 years. In part due to improved ethical and regulatory frameworks to ensure participant safety, the use of human challenge has increased over the last few decades.

Contemporary uses of these types of study have generated key data to expedite and streamline vaccine and therapeutics development and support broader public health policy decision-making. Modern research methodologies and high through-put laboratory techniques allow the interrogation of human host responses and the underlying molecular genomic basis for these on a level previously impossible to reach. Additionally, human challenge studies can provide more specific host relevant data than animal models, either replacing or providing a bridge for translation of basic scientific discoveries.

This article, reviews the contemporary literature relating to the aims, design and conduct of human challenge studies, including discussion of clinical, scientific and regulatory considerations.

## ABSTRACT

Since the 18<sup>th</sup> century a wealth of knowledge regarding infectious disease pathogenesis, prevention and treatment has been accumulated by performing infection challenges in human subjects. With a recent renaissance in popularity a multiplicity of possible study designs that emphasise participant safety now exist aiming to address a broad variety of medical and scientific questions. Studies cover the spectrum from basic science to applied translational research and product development, incorporating diagnostics, vaccines and therapeutics discovery and evaluation.

Herein we draw on the published literature relating to human challenge studies and consider contemporary views from the expert community relating to the progress, value and possible future developments of such work. In the context of the scientific and public health value of challenge studies, we attempt to summarize the study design and implementation considerations vital to performing such complex research. Specifically, questions regarding participant safety, effective study design, volunteer selection and consent processes are addressed. Furthermore, technical issues and limitations relating to choice of challenge agent, route of infection, inoculum size, endpoint criteria, and safety monitoring are highlighted using examples from various models recently and currently used.

## INTRODUCTION

Experimental human challenge by deliberate exposure of participants to infectious substances has been an important research approach for nearly 300 years. While broadly similar in process, the scientific rationale for this type of research is diverse, ranging from conclusively demonstrating Koch's disease causation postulates to the efficacy testing of novel treatments or vaccines in a highly-controlled infection setting.

Since inception, challenge studies have been ethically and logistically demanding, not least in directly flouting the Hippocratic principle of '*Primum non nocere*' (first do no harm). The perceived acceptability of such 'risky' research and thus the availability of volunteers, a study site and a suitable challenge agent contribute towards the complex ethical and logistical deliberations necessary before performing such work. This complexity is amplified by subtle nuance in the 'actual' versus 'perceived' risk these studies pose for participants, investigators, and sponsors. The often murky historical context of performing deliberate infection studies must also not be overlooked. Many early studies performed clearly and deliberately breached both contemporary ethical and moral standards for the purposes of individual or organisational gain.<sup>1,2</sup>

In the late 20<sup>th</sup> and early 21<sup>st</sup> century enhanced research accountability, robust ethical and regulatory scrutiny and, in some cases, the provision of consensus frameworks for performing human challenge has led to resurgence in popularity.<sup>3-5</sup> Multiple adjectives are now used to describe the 'challenge' process including experimental, artificial or induced in addition to the terms controlled human infection or deliberate exposure.

In this review, we summarise some of the scientific, technical and logistic considerations pertinent to the design of human challenge studies. The ethical and legal justification for performing such research is beyond the scope of this review, but has been reviewed elsewhere.<sup>1,6,7</sup>

## REASONS TO CHALLENGE

Since the 18<sup>th</sup> century, deliberate infection of humans with known or putative pathogenic substances or exposure to presumably infectious persons has been used to further scientific, clinical and public health enquiry. The first true challenge with an infectious agent was performed to assess the safety of variolation for its use in preventing smallpox.<sup>8</sup> This work was emulated some years later by Jenner to demonstrate the efficacy of vaccination against smallpox infection.<sup>9</sup> Today, challenge studies continue to provide a valuable role in the assessment of protective immunity by allowing investigators to

directly measure the efficacy of vaccine candidates against the infection of interest (Supplementary Table S1, Figure 1). Assessment of natural infection-derived protection has also been studied in several pathogen models including *Salmonella* Typhi, enterotoxigenic *Escherichia coli* (ETEC), *Giardia lamblia* and a recently revived model of leishmanisation (*Leishmania major*).<sup>10-13</sup> Usefully, measures of vaccine efficacy found in human challenge studies have been found to relate closely to those obtained in subsequent field trials for many, but not all infections. The *Plasmodium falciparum* vaccine candidate RTS,S for example, has demonstrated efficacy of approximately 30-50% (sterile protection) using the controlled human malaria infection (CHMI) model,<sup>3,14,15</sup> a level of protection subsequently borne out in phase II/III field-trials.<sup>15-17</sup>

Key to the resurgence of challenge studies for vaccine evaluation is the opportunity for investigators to up- or down-select potential vaccine candidates at an early stage, avoiding costly field-testing phases for non-efficacious prototype development.<sup>18</sup> Challenge studies also play an important role in “bridging” data/results from one setting, population or infection strain to another. This was exemplified in demonstrating the protection by oral cholera vaccine CVD 103-HgR against the 01 El Tor Inaba biotype of *Vibrio cholerae* in a human model.<sup>19</sup> In combination with previously demonstrated efficacy against classical biotype infection,<sup>20</sup> these data were accepted by regulatory agencies in several regions towards the licensure of CVD 103-HgR for travellers.<sup>21,22</sup>

Similarly, proof-of-concept efficacy studies for candidate therapeutics can be efficiently undertaken in experimentally infected volunteers, as was done for the development of both classes of currently available anti-influenza drugs.<sup>23</sup> An additional application relevant to both vaccine and therapeutic studies include the selection of dosing or administration regimes for subsequent field-testing. This was performed for the neuraminidase inhibitor oseltamavir in treating experimental influenza infection, for example, and for a variety of investigational therapeutics in the CHMI and *Shigella flexneri* models.<sup>23-26</sup>

Challenge studies may produce invaluable laboratory data suggesting possible correlates of protection or immunological pathways amenable to augmenting vaccine efficacy. Recently, the CHMI model has refuted the role of a strong cellular immune response to viral-vectored vaccination in affecting parasite growth rates, for example, suggesting that future vaccines targeting blood-stage infection should be directed towards generating high antibody titres.<sup>27</sup>

Human challenge studies offer the most direct approach to demonstrating Koch’s postulates of disease causation for a putative pathogen. These studies include well-known

'self-challenge' experiments, including those by John Hunter and more recently by Barry Marshall in demonstrating the virulence of *H. pylori* in causing acute gastritis.<sup>28-31</sup> Historically, significant work performed at the MRC Common Cold Unit identified rhinovirus as the predominant cause of the common cold.<sup>32</sup> In addition to discovering methods for rhinovirus culture *in vitro*, challenge studies also allowed identification of the prophylactic benefit of intranasal interferon in preventing rhinovirus colds (reviewed in 33),<sup>34,35</sup> a finding later confirmed in household-based field trials.<sup>36,37</sup> More recent questions regarding disease aetiology include the search for putative virulence factors and mechanisms underlying host susceptibility (Supplementary Table 1). A *Neisseria gonorrhoea* challenge model directly assessed the potential virulence of IgA1 protease by challenging male volunteers with a wild-type or IgA1 protease-deficient strains.<sup>38</sup> In a different approach, monitoring antigen variation by *G. lamblia* during challenge has provided some indication as to how this pathogen may evade the host immune response.<sup>39</sup> Extensive investigations have also been performed using a skin-inoculation model of *Haemophilus ducreyi* infection, assessing the effects of pili, cytolethal distending toxin (CDT) or haemolysin deficiency on the rate of pustule formation.<sup>40,41</sup>

A major use of human challenge studies has been to investigate those host-restricted pathogens for which no suitable animal model exists. In several cases, animal and non-human primate (NHP) models are available, however the illness caused does not sufficiently replicate human infection. In studies of dengue infection for example, the virus may be used to infect NHPs leading to viral replication and an antibody response, but without the development of clinical disease.<sup>42-44</sup> Furthermore, interpretation of the disease endpoints or surrogate measures of protection obtained in animal models and how they relate to human responses is often poorly understood. Lack of specificity and interpretability of animal data are major drivers for the development of new human models, including an intradermal BCG model to identify correlates of anti-mycobacterial immunity in humans that are relevant to *Mycobacterium tuberculosis* infection.<sup>45,46</sup>

In many challenge models, volunteers are permitted to continue with their normal daily activities potentially allowing even more 'human-specific' data to be recorded.<sup>47</sup> The role of 'normal' activity has been explored even further in ongoing influenza challenge studies, in which human-human interactions are being explored to investigate the behavioural factors underlying virus transmission and evaluate methods to prevent cross-infection.<sup>48,49</sup> In this quarantine environment, almost every detail of natural interaction (e.g. proximity and frequency) in addition to the environment (humidity, temperature

etc.) may be controlled, permitting replicable data to be collected with a precision and level of detail that could not be achieved using alternate methods or 'real-life' settings.<sup>49</sup>

Frequently, the idiosyncratic nature of infectious disease epidemiology and of emerging epidemics requires urgent data to be collected regarding likely vaccine or treatment efficacy. Established human challenge models may be used to provide these initial proof-of-concept data, as has been proposed for the development and licensing of a universal influenza vaccine.<sup>50</sup> Alternatively, some infections occur so infrequently in nature that study of therapeutic or control strategies is not feasible using naturally occurring cases. This applies to sporadic, seasonal infections including *Bordetella pertussis*,<sup>51</sup> but also holds true for many potential biological warfare agents, including *Francisella tularensis*, *Coxiella burnetii* and *Rickettsia rickettsii*.<sup>52</sup> Concern over the deliberate release of these pathogens places research into the prevention and treatment of such infections as a high priority.<sup>53</sup> In addition to the direct assessment of treatment effects/benefits and the opportunity to glean useful pharmacokinetic/pharmacodynamic data from longitudinal volunteer monitoring, challenge studies may also be used to validate management algorithms. For example, there are no specific treatments currently available for dengue infection, however using a human challenge model, a standardised care pathway in addition to predefined endpoints, has been shown to be effective in preventing severe infection.<sup>43,54,55</sup>

Contemporary challenge studies are exploring the full extent of the 'hygiene hypothesis' by studying the potential therapeutic immune-modulatory effects of helminth infection on various autoimmune and allergic diseases. Exciting development in this regard promise has been shown using hookworm (*Necator americanus*) treatment for asthma,<sup>56</sup> or coeliac disease,<sup>57</sup> (a study to investigate effects on multiple sclerosis, WIRMS,<sup>58</sup> is still in progress) and *Trichuris suis* therapy for Crohn's disease.<sup>59</sup>

## STUDY DESIGN & SETTING

Important considerations in designing human challenge studies include the setting in which the study is to be performed and the methods used for screening and volunteer selection.

### *Inpatient versus ambulant design*

Human challenge studies are performed by academic and commercial organisations in different settings, the choice of which is governed primarily by participant safety, pathogen transmissibility and virulence, as well as infection control considerations.<sup>60</sup>

Confinement of participants to hospital wards or research units is clearly required if there is a risk of transmitting potentially virulent pathogens to the environment or the public. Alternatively, common pathogens likely to be encountered under natural conditions, e.g., rhinoviruses, can be studied outside of isolation facilities. Conversely, participants may themselves need isolating to prevent acquisition of naturally encountered infection, which may alter the scientific interpretation of data obtained.<sup>60</sup> Legal requirements in some countries may enforce isolation of participants until treatment has been commenced for specific infections (typhoid or cholera challenge in the US),<sup>61</sup> or may mandate reporting to relevant public health authorities, despite occurrence in an artificial setting (such as for gonorrhoea).<sup>47</sup> Logistic ramifications of such legislation may include the prolonged isolation of participants with infections such as typhoid (for up to 32 days), due to the lengthy incubation period involved,<sup>62</sup> or declaration to health insurance providers.

Despite the high cost and sometimes greater difficulty in identifying suitable volunteers, there are however multiple advantages to using an inpatient design. These include the ability to collect detailed observational data, more accurate clinical sampling and to initiate adjunctive therapy and treatment as soon as it is required (e.g., oral or intravenous fluid replacement),<sup>61</sup> and to minimize the chance of inadvertent transmission of the challenge pathogen to contacts.

Many early challenge studies were performed using prisoners<sup>52</sup>, residents of custodial institutions<sup>63</sup>, or military personnel.<sup>64</sup> While use of these populations is now considered unacceptable (custodial institutions) or is generally considered unacceptable due to ethical reasons, this has led to a keen interest in assessing participant's experiences of taking part in such studies. Progressive work performed at the Common Cold Unit, for example, advanced understanding of the effect of confinement and personal stress on the immune response and thus susceptibility to colds in healthy human challenge study participants.<sup>33,65,66</sup>

With the appropriate precautions being taken, contemporary challenge studies are often performed using ambulant outpatient volunteers. These precautions may include notifying close household members of the volunteer's participation, having a 24-hour on-call medical support team, and frequent outpatient review visits. In current *N. gonorrhoea* challenge studies for example, participants are required to return to the study site each night to minimise the risk of transmission during the high-risk hours of darkness!<sup>47</sup>



### ***Examples of specific challenge study settings***

As the parameters within which modern human challenge studies may be safely performed have emerged, attempts are being made to make results of these studies even more relevant to the target patient/at-risk population. These studies include performing challenge in participants with known comorbid disease processes, for example rhinovirus challenge of patients with asthma,<sup>67</sup> or chronic obstructive pulmonary disease.<sup>68,69</sup> These types of studies require additional levels of medical vigilance to be taken to address the potential deterioration of the participant's clinical condition.

There is scientific rationale for performing studies of disease susceptibility, vaccine efficacy and treatment interventions in appropriate endemic settings. For example, both *V. cholerae* and *Shigella sonnei* human challenge models have been established in Thailand by successful collaborations between Thai and US investigators.<sup>70-72</sup> While demonstrating feasibility, these studies have highlighted the difficulties in screening for an immunologically naïve participants in endemic settings. This may lead to the requirement for higher challenge doses to replicate the attack rates (ARs) or illness severities seen in North American volunteers.<sup>70,73</sup> These studies also highlight the importance of translating illness definitions/endpoints between studies and the benefit of having frozen challenge strains that may be shared between sites and investigators (further discussed below).<sup>71</sup>

### ***Volunteer screening & selection***

Careful volunteer selection is important both for individual safety and the integrity of the scientific question being addressed. Thus, individuals enrolling into challenge studies undergo a rigorous, often multi-stage screening process prior to participation (see Table 1).

In many studies, participants are screened and excluded if pre-existing antibody titres to the organism being investigated are found. In influenza challenge for example, pre-existing immunity not measured by specific antibody screening, and present in all adult volunteers as a result of prior influenza infection, may significantly affect responses to challenge.<sup>50,74</sup>

Overly careful selection of healthy volunteers for challenge studies may affect the applicability of the data generated, however. For example, healthy, male, adult volunteers without, serological evidence of exposure to a particular infection, may only poorly represent the host responses to wild-type infection seen in the general population. Equally, environmental factors including temperature and humidity or the nature of

currently circulating strain types may also be critical, particularly for respiratory viruses.<sup>75</sup>

Finally, financial payments are often made to volunteers engaged in these types of time and labour-intensive studies and while fraught with ethical and moral issue, this should be commensurate with the study demands without being coercive. To-date no generally agreed standards specific to experimental human challenge studies are recognized for the consent or compensation levels paid and decisions are the purview of ethical review.<sup>76,77</sup>

**Table 1: Examples of some specific screening considerations for certain challenge types.**

Challenge agent	Screening consideration(s)	Screening method
<i>Cryptosporidium</i> sp. <sup>78</sup>	Protective effect of pre-existing antibodies to <i>Cryptosporidium parvum</i> .	Blood test
<i>Campylobacter jejuni</i> <sup>79</sup>	Protective effect of pre-existing antibody to infection and risk of GBS or reactive arthritis	Blood tests and elicited family history
<i>Salmonella</i> Typhi <sup>80</sup>	Chronic carriage – found in approximately 3.5% of healthy individuals in endemic settings <sup>81</sup>	Ultrasound scan and exclusion of those with stones/history of gallbladder disease
Norovirus <sup>82,83</sup>	Secretor-negative persons (non-functional FUT2 gene) are resistant to Norwalk virus infection	Exclusion by detection of fucosyltransferase 2 in saliva
Rhinovirus <sup>67</sup>	Strain specific neutralizing antibodies	Blood test
Influenza <sup>48,74</sup>	Neutralizing and hemagglutination-inhibition antibodies	Blood test
BCG <sup>84</sup>	Screening for latent tuberculosis.	IFN $\gamma$ T-cell ELISPOT <sup>85</sup>
Dengue <sup>42,54</sup>	For vaccine trial – neutralizing antibody response had to be detectable post-vaccination to be eligible for challenge. Screening control subjects for flavivirus seronegativity.	Blood test
<i>Vibrio cholerae</i> <sup>86</sup>	Blood group ‘O’ conveys susceptibility to more frequent and severe diarrhoea/disease	Exclusion (or selective inclusion) by blood group test
<i>Shigella</i> sp. <sup>87</sup>	HLA B27 carriers are at higher risk of inflammatory reactive arthritis after infection/exposure Occupational food handlers are not eligible	Laboratory tests and medical history

## ***Safety considerations***

Participant, staff, public and environmental safety are clear primary concerns in performing deliberate infection studies with pathogenic agents.<sup>5-7,62</sup>

Core to the findings of the Nuremberg Trials, which investigated the unethical medical procedures carried out during WWII, and the subsequent Declaration of Helsinki is that human subjects must consent to participation in research.<sup>88</sup> Ensuring that participants understand the full implications of participation in such studies can be a complex procedure, particularly in challenge studies, when several stages of participation may be involved. Methods to ensure that informed consent has been given may include separating the consent procedures for vaccination and challenge (e.g., norovirus VLP vaccine and challenge),<sup>83</sup> or repeating the consent procedure prior to challenge (e.g. Oxford typhoid and malaria models).<sup>80,89,90</sup> Importantly, assessment of the participant's understanding of consent can be gauged by use of questionnaires or written 'exams' (e.g., Centre for Vaccine Development (CVD) including *E. coli* and cholera challenge models),<sup>7,19,91</sup> and obtaining the participant's primary care practitioner's approval for enrolment in the study (e.g. Oxford malaria and typhoid models).<sup>89,92</sup>

After fully informed consent has been provided, participant safety assessment continues with vigorous medical screening procedures, which often involves obtaining the participants medical or vaccination records and/or psychological assessment, in addition to the specific infection model requirements (Table 1). Participant selection and assessment criteria, while broadly similar between studies, are clearly dynamic and affected by new data and events occurring in challenge models. Investigation of an episode of myocarditis after influenza B challenge occurring in a single study participant, for example, resulted in heightened screening for pre-existing cardiac conditions across the influenza challenge field.<sup>93</sup> The participant made a full recovery; similar events have been reported in the CHMI model similarly strengthening the screening protocols used.<sup>94,95</sup> Staff safety while performing challenge and in handling potentially infectious samples is also important, and risks can be reduced by suitable risk-assessment, staff vaccination (when relevant) and infection control services. Management of the risk of transmission amongst the general public when using ambulatory challenge model designs involves careful risk assessments of the challenge strains used and potential exclusion of participants posing an increased risk of transmission (e.g. food handlers in enteric challenge models).

Together with these human factors, various microbiological factors are equally important to ensuring participant safety. These include choice of challenge strain (balancing

requirement for virulence, thus ensuring a clinically applicable model, with manageable symptom profile and rapid response to therapy), route of delivery (described above), treatment of infection/symptoms and confirmation of infection clearance/resolution. Examples of the pathogen-specific issues include the selection and use of modified strains such as CagA- strains of *H. pylori* (given the association between CagA and gastric cancer),<sup>96</sup> or the prevention of *Campylobacter* recrudescence after receipt of antibiotics.<sup>97</sup> Occasionally this close attention to minutiae results in unique observations, such as the *in vivo* documentation of antibiotic resistance developing.<sup>98</sup> Use of antibiotic resistant bacterial strains to perform challenge is currently beyond most ethical boundaries and is considered unsafe, however is a further example of how removed challenge models may be from most real-life settings.

## CHALLENGE AGENT

Crucial to the setting up of a human challenge study is the choice and availability of a suitable challenge strain. The repertoire of currently available challenge strains is quite narrow due to the costs associated with GMP production, type of pathogen and available culture methods (e.g., norovirus) and potential regulatory hurdles. Furthermore, the necessity of thorough strain characterization including genetic sequencing, antibody susceptibility testing, identification of ARs and extensive pre-clinical experience significantly limits the expansion of potential strain repositories. Thus, often researchers are required to rely on historical strains (such as the typhoid Quail strain<sup>89</sup>). Collaborative efforts will hopefully address these issues and grow a reasonable repository of challenge strains (Box 1). Which strain is chosen may have significant implications for the study findings, as variations in virulence, transmissibility and genetic stability may alter the infection profile and risk to participants considerably (Table 2).

This has been demonstrated in a human model of *Campylobacter jejuni* infection.<sup>99</sup> There are also specific biological issues to consider with certain challenge agent selection, including for example the association between certain *C. jejuni* LPS-ganglioside mimicry and the development of Guillain-Barre syndrome.<sup>100</sup> In vaccine studies, strain choice may be guided by the suggested mechanism of vaccine protection (for example, antibody or cell mediated immune response) and whether homologous or heterologous protection is to be demonstrated. One successful strategy used by many vaccine studies as the first step towards vaccine development, is to use an attenuated strain for vaccination and then the same, non-attenuated strain for challenge, for example tularaemia LVS (Live Vaccine Strain),<sup>101</sup> or for live-attenuated intranasal influenza vaccine in infants.<sup>102</sup> While not

meeting the safety profile of a vaccine, use of reactogenic strains may be considered to induce sufficient symptoms to be in keeping with the required infection profile, for example the dengue 1 vaccine strain 45AZ5.<sup>54,103</sup>

Strain availability may vary considerably due to complexities in strain stability. Norovirus, for example, is difficult to culture and requires a fresh isolate prior to challenge.<sup>104</sup> Standardisation of challenge models across geographically distant sites is also often required, so that research findings may be replicated and verified, to compare competing vaccine/treatment candidates and to broaden the opportunities for participant recruitment. For this to be effective, and to achieve consistent challenge profiles, the availability of frozen strains has greatly helped various models including cholera, typhoid and malaria challenge.<sup>61,80,89,105</sup>

**Table 2: Specific considerations given to the choice of specific strains of pathogens for use in human challenge studies**

Challenge model, strains	Consideration/concern/advantages/disadvantages
Enterotoxigenic <i>Escherichia coli</i> , strain H10407	Reliably produces more severe diarrhoeal responses when compared to other strains assessed – ‘most virulent challenge strain’ producing more loose stools and higher volume diarrhoea, but well characterised and therefore the ‘benchmark’ strain. <sup>11,106</sup>
<i>Helicobacter pylori</i> , strain BCM100 (ATCC reg)	CagA positivity – associated with the development of stomach cancer. Thus initial challenges carried out using CagA- strains, however most common strains are CagA+. <sup>96</sup> All strains used are antibiotic-susceptible.
<i>Cryptosporidium parvum</i> (Iowa, VCP, TAMU, Moredum), <i>C. hominis</i> (TV 502), <i>C. meleagridis</i> (TU1887)	<i>C. hominis</i> more frequently associated with asymptomatic infection than <i>C. parvum</i> Purified oocysts from infection calf faeces; administered in gelatin capsules. <sup>107</sup>
Norovirus strains G1.1, G2.2, G2.4 <sup>82</sup>	Challenge virus derived from stool filtrate – donor followed-up for >10years (syphilis, HCV, HBV, HIV, HTLV). Fresh isolate needed due to lack of culture method.
<i>Giardia lamblia</i>	GS/M – which was the strain considered infective; strain Isr was used as control as none of the participants developed signs consistent with giardiasis. <sup>12</sup>
<i>Vibrio cholera</i> 01, Tor Inaba strain N16961 <i>Vibrio cholera</i> 0139	Pre-frozen strains which have demonstrated standardised level of infection. <sup>61,86</sup>
<i>Salmonella</i> Typhi, Quail strain	Fresh isolate with documented virulence through transmission to family members, reliable Vi expression; frozen GMP manufactured Quail strain, antibiotic sensitive. <sup>80,108</sup>
<i>Shigella flexneri</i> 2a strain 2457T	Shiga toxin negative, genetically stable, antibiotic sensitive. <sup>109,110</sup>
<i>Mycobacterium bovis</i> BCG as a surrogate for <i>Mycobacterium tuberculosis</i>	BCG challenges are being developed to assess as ‘attenuated’ TB challenge model. <sup>45</sup>

Strain availability is also dictated by the various jurisdictional requirements of the country or region in which the study is to be performed. Challenge agents in the USA are designated as pharmacologically active agents and their release therefore requires Investigational New Drug (IND) approval by the FDA.<sup>111</sup> In contrast, challenge agents used in the UK are not classed as pharmaceutical products under the European Clinical Trials Directive (ECTD 2001/20/EC) and therefore regulatory approval (from the **Medicines and Healthcare products Regulatory Agency**) is not currently required prior to their use.<sup>5,105</sup> **Deliberate attenuation or genetic alteration of strains requires additional approvals for genetically modified organism (GMO) release in most regions, as well as specific permissions for contained use or deliberate release.**

The different approval processes in the USA and Northern Europe and between the different countries of the European Union, means that international multi-centre studies are logistically complex and expensive at the present time.<sup>86</sup> In several areas, public-private partnerships are starting to address this problem, by producing strain libraries for both academic and industry-sponsored study use, and by larger-scale production of standardised, well-characterised, pre-frozen strains for 'off-the-shelf-use'.<sup>50,86,105</sup>

## **DOSE & ROUTE OF INFECTION**

How to actually perform challenge once inocula are available is frequently a source of further deliberation, as there are many associated safety, scientific and practical considerations. Replication of natural exposure is often scientifically the most relevant but may not be physically or logistically practical or considered to be safe. Replicating natural infection requires knowledge of the exposure dose, which, if known, is often lower in the immunologically-naïve highly selected volunteer cohort.<sup>22,70,112</sup> The natural attack rate (AR) of most infections is relatively low and/or often associated with mild or subclinical infections detected by serologic parameters. Consequently, in order to study sufficient numbers of exposed and infected individuals, particularly those with clinical manifestations, larger numbers of study participants would be required. Experimental challenges therefore often use larger, 'non-natural' exposure doses, to induce a high AR, and to make more efficient use of available resources. In most infections, higher doses lead to a shorter incubation period.<sup>113</sup> While higher ARs often allow for smaller study cohorts, they may overwhelm vaccine- or infection-derived protection, cause more severe infection symptoms or make the experimental findings less physiologically relevant.<sup>73</sup> This has motivated many investigators to lower the challenge dose used in enteric challenge studies. Methods employed include the use of sodium bicarbonate solution to



reduce the effects of gastric acidity on bacteria viability in the *Shigella* or typhoid challenge models.<sup>73,80,89</sup>

Reproducing the route of natural exposure also has an impact on the nature of disease caused and the corresponding host response being studied (Table 3). This is a significant limitation of respiratory virus challenge studies, as in general, aerosol challenge to the lower respiratory tract leads to more severe illness with a higher risk of complications.<sup>114,115</sup> Nasal instillation is therefore frequently used, and this leads to the necessity of nasal dosing of topically delivered therapeutics and also in the case of influenza, an illness profile that differs considerably from natural disease. For nasopharyngeal bacterial challenges the endpoints measured include colonisation by the instilled organism.<sup>116,117</sup> An exception to this upper airway approach being the transdermal BCG challenge study described above.<sup>45,118,119</sup> The dermal route is advantageous as direct inspection of the site can be easily performed and biopsies to monitor the immune response may be easily obtained.<sup>45,118</sup> Interesting observations have been made investigating the route of infection in leishmania challenges. While subcutaneous inoculation led to lesion formation it appears that parasites transmitted by sandflies are more efficient in causing infection, indicating that the parasite potentially undergoes developmental changes within the sandfly.<sup>120</sup> Vectored versus direct subcutaneous or intravenous infection with malaria,<sup>105,121</sup> dengue,<sup>122</sup> and filaria,<sup>105,123</sup> have also been explored in some depth in efforts to standardise challenge delivery in addition to understanding disease mechanisms.

**Table 3: Examples of considerations specific to the type and route of infection being used**

Route/ pathogen	Consideration	Approach taken
Respiratory – viruses including Influenza, RSV	Risk of more severe lower respiratory tract infection with aerosolised infection. Person-to-person transmission and the effect of behaviour/habits.	Nasal instillation of challenge agent – demonstrated effectiveness in assessment of vaccines and therapeutics. <sup>124</sup> Exposure of non-immune individuals to recently challenged and confirmed infected study participants. <sup>48</sup>
Nasopharyngeal – including <i>S. pneumonia</i>	Risk of invasive disease including pneumonia or meningitis.	Measurement of carriage as a surrogate endpoint for conjugate vaccine efficacy. <sup>117</sup>
Enteric – <i>S. Typhi</i> , <i>Vibrio cholerae</i> , ETEC, <i>Shigella</i> , <i>H. pylori</i>	Gastric acid milieu acting as a broad and effective non-specific barrier to infection.	Use of acid suppressants both prior to challenge (milk, NaHCO <sub>3</sub> , proton pump inhibitors (famotidine – <i>H. pylori</i> ), rice-based buffer solution [Ceravac - ETEC]) and as the carrier solution (milk, NaHCO <sub>3</sub> ) – use of NaHCO <sub>3</sub> has been shown in multiple studies to require a lower challenge inoculum to produce the same attack rate and to produce a ‘smoother’ more consistent attack rate. <sup>11,61,73,96,125</sup> Variations in duration of prior fasting, co-administration with NaHCO <sub>3</sub> solution and assorted vehicles including apple sauce. <sup>106</sup> 3hrs of fasting and NaHCO <sub>3</sub> solution as vehicle. <sup>86</sup>
Mosquito bite vs. blood stage challenge	Transdermal infection/administration of infected erythrocytes.	Sporozoite vs. challenge with infected erythrocytes – depending of immune response of interest (i.e. pre-erythrocytic immunity), time to patency etc. <sup>126,127</sup>
Hookworm infection	Transdermal infection.	Administered cutaneously to replicate natural infection – causing a temporary rash or itching. <sup>128</sup>
Gonococcal infection	-	Instillation into anterior urethra. <sup>47</sup>

## INFECTION ENDPOINTS/CRITERIA

Choice of endpoints in challenge studies is critical to both minimise the risk to participants (both the number exposed and severity of infection caused) and to ensure that the clinically and/or scientifically useful outcomes are assessed.<sup>129</sup> Dependent on the type of study being performed, the endpoint may be diagnosis of infection, as measured by a diagnostic test, carriage or infection by specific strain types, the development of infection signs and symptoms or assessment of infection severity. While predefined endpoint criteria are required, these have considerable impact on ARs as has been shown in a retrospective analysis of the Maryland typhoid challenge studies.<sup>113</sup> Depending on the pathogen, quantitative illness measures (e.g., fever, stool volumes nasal mucus weights, middle ear pressures) can provide objective outcome data.<sup>130</sup> Different endpoint criteria allow for more detailed assessment of model outcomes, i.e. distinguish between 'infected' and 'infected/diseased' participants in order to further stratify challenge 'take', or assess intervention impact on symptomatic and asymptomatic infections.<sup>83</sup> The challenge study endpoint, which is also generally the point at which rescue treatment (e.g. antibiotics) is initiated, is often composed of several clinical, microbiological/serological components. While potentially making studies safer for participants by allowing multiple different triggers for rescue treatment initiation, composite endpoints tend to be less well-established and therefore open to different interpretation by investigators and study sites (Table 4).

**Table 4: Examples of endpoints used in human challenge studies**

Model	Clinical Endpoint	Microbiological Endpoint	Other
Cholera <sup>a</sup> <sub>61,86</sub>	Diarrhoea (purge) <sup>a</sup>	Stool culture	-
Typhoid – Maryland <sup>b</sup> <sub>113</sub>	Temperature	-	-
Typhoid – Oxford <sup>b</sup> <sub>80</sub>	Temperature ( $\geq 38^{\circ}\text{C}$ for $\geq 12$ hrs)	Blood culture	-
ETEC <sub>106</sub>	Diarrhoea using stool grading and symptoms as severity score.	Quantitative stool culture (early shedding predictive of subsequent disease)	-
Norovirus <sub>104,131</sub>	Gastroenteritis (Infection/disease): - >200g of watery feces - 1 vomiting episode§ - > 3 loose or watery stools	Infection: Faecal shedding (stool PCR)	Infection: Serology – 4fold increase in antibody titres
<i>H. pylori</i> <sub>132</sub>	-	Culture	Histology, rapid urease test, urease breath test <sup>b</sup>
Influenza <sup>a</sup> <sub>49</sub>	Temperature, symptom scores	Serology, culture and PCR (RNA, infectious virus) on nasopharyngeal washes	-
Malaria <sub>133</sub>	-	-	Parasitaemia (qPCR), microscopy
<i>Shigella</i> <sub>73,87</sub>	Diarrhoea <sup>c</sup> & dysentery symptoms, temperature (2 oral temperatures $\geq 100^{\circ}\text{F}$ at least 5 minutes apart)	-	-

<sup>a</sup> Cholera – stool weight used to grade illness severity; >3Kg= moderate, >5Kg = severe

<sup>b</sup> *H. pylori* – composite endpoint score, 0=no infection, infection severity graded 1-4

<sup>c</sup> *Shigella* – diarrhoea volume used to grade illness severity

Nb., the same definitions of ‘diarrhoea’ are used for many of the enteric challenge studies including those performed at the Centre for Vaccine Development (Maryland), cholera, ETEC and *Shigella* models.

§ plus either abdominal cramps/pain, nausea, bloating, loose stools, temperature ( $>99.7^{\circ}\text{F}$ ), myalgia, or headache.

Choice of endpoints is not always straightforward however, as host-responses vary considerably between challenged individuals. Relating to a multitude of factors, including host susceptibility, genetic background, prior exposure and challenge agent variation, some challenge agents produce a relatively variable clinical presentation. This requires flexibility in initiation of treatment and continual close monitoring of participants undergoing challenge (e.g., shigellosis, cholera).<sup>109</sup>

Using the development of infection as the endpoint in challenge studies also provides a unique opportunity to identify and validate novel biomarkers and diagnostic tests. The availability of pre-challenge samples means that baseline susceptibility factors can be interrogated, as has successfully been demonstrated in the norovirus challenge study.<sup>131</sup> Furthermore, subsequent samples collected after challenge allows the direct investigation and assessment of putative correlates of protection following either infection or vaccination.<sup>80,134,135</sup> In addition, the availability of baseline samples with knowledge of subsequent outcome after challenge, allows the direct investigation and assessment of putative correlates of protection following either infection or vaccination (Table S1).<sup>80</sup>

## DISCUSSION

In this overview we have described the renaissance of human microbial challenge studies in the 21<sup>st</sup> century. Recent major advances in the understanding of disease mechanisms and host responses, coupled with more affordable, high-throughput technologies have reignited the opportunities for obtaining informative, predictive, and often novel data from human challenge studies. Whilst the historical moral and ethical challenges remain and should remain under constant inspection and discussion, never has this type of research been more feasible and valuable.

When performed in the appropriate manner, challenge studies have an excellent safety profile, likely due to careful selection of participants, adherence with study procedures and to heightened investigator vigilance. The range of challenge models currently in use exceeds twenty, while the number of volunteers who have safely undergone infection challenge runs into the tens of thousands.

Furthermore, it is clear that in many cases, whilst operating within the appropriate ethical committee mandate, institutions and investigators work in relative isolation or operate solely in disease- or pathogen-specific areas. Coordination and communication among these different areas of study and models using a horizontal approach by sharing

protocols and common experiences could greatly benefit the field. This in turn may lead to standardisation of reporting procedures, not only to ethics review boards and regulatory agencies but also in the publication of study results.

There is a general shortage of challenge strains whether through the lack of experience or sufficient safety profile with a particular, recently isolated strain, or due to the specific technical issues in strain isolation/production, characterisation and storage. Specific challenge agents are particularly problematic or lacking, including *H. pylori*, RSV, noroviruses, and a sufficiently broad range of influenza strains. The narrow repertoire and the often attenuated nature of challenge strains used potentially limits the adequate extrapolation of study findings into clinical field settings and ignores the potential effects of strain-to-strain variation. Collaboration and sharing of challenge strains among institutions and study sites may not only effectively address this issue, but also promote a collegial atmosphere supportive of conducting such expensive pre-clinical safety and dose-ranging studies. This has been very successful in specific public-private partnerships including the provision of cryopreserved *P. falciparum* strains, frozen *Vibrio cholerae* and *Salmonella* Typhi Quail's strain.<sup>89,105,136</sup> The availability of challenge strains, which may cost in excess of \$200,000 to manufacture to GMP standards, allows new sites to consider performing this type of research including those in more resource-limited settings. For example, cholera and *Shigella* studies have been undertaken in Thailand, and several African centres recently started to perform CHMI studies.

Central to the on-going success of human challenge work is the careful balance between close regulation of safety and ethical processes with the freedom to make important scientific advances. While some experimental infection has not always been performed ethically, it has encouraged debate and led many of the advancements resulting in the ethical principles now held central to clinical trial work. Despite a sometimes-uncertain prospect in the past, human challenge studies are positioned to have a major impact on medicine and global health for a 3<sup>rd</sup> century.

Several examples of such research have made some significant advances in clinical and scientific research. These range from proof of concept that challenge studies are useful in testing vaccine candidates such as reproducing field efficacy rates for the malaria vaccine RTS,S. A fascinating outlook and potentially significant stride forward in therapeutics research is the use of the hookworm challenge model in potentially treating multiple sclerosis. However successful challenge studies have been in the post-WWII era, the primary focus on participant safety must continue to underpin possible major advances

through use of human challenge studies. Both Jenner and his first challenge volunteer, James Phipps, would be astonished by the impact of their contribution in this field.

### **Search strategy and selection criteria**

This manuscript is based on the search strategy detailed below and on the topics discussed at the meeting entitled 'Controlled Human Infection Studies in The Development of Vaccines and Therapeutics' (Jesus College, Cambridge, January 2013) and is intended to give an overview of the progress and challenges within the field of human challenge studies. Therefore the specific topics detailed in this manuscript are reviewed in the context of recent challenge studies discussed in the literature.

The search strategy consisted of PUBMED searches for the following terms:

'infection\* in volunteers'; 'human volunteer\* challenge\*'; 'human challenge model\*'; 'volunteer challenge stud\*'; 'human challenge stud\*'; 'variolation\*'; 'human infection model\*'; 'human\* experimentally infected'; 'experimental human infection\*'; 'experimental infection\* in human\*'; 'controlled human infection\*'; 'human BCG challenge'.

The search was conducted for articles added between 1946 until present in both databases. Articles written in other languages than English, reporting on challenges of non-human animal models or challenges not using infectious agents were excluded.

Broader searches were also performed using pubmed to identify articles reporting events prior to 1946 and dating back to the 17<sup>th</sup> century. Further studies were identified from references of articles identified by the search strategy. Finally, further unpublished data and additional references were collected during a recent meeting entitled 'Controlled Human Infection Studies in The Development of Vaccines and Therapeutics' held in at Jesus College, Cambridge, UK, January 2013.



## **Acknowledgement**

The authors acknowledge the Wellcome Trust, London, the British Society for Immunology, London and the Jenner Institute, Oxford for their generous support which made this meeting possible. TCD, AJP and CJB are supported by the NIHR Oxford Biomedical Research Centre (Translational Research Fellowship to TCD). CJB is supported by the European Union (FP7) as a Marie Curie Research Fellow. AJP and AVSH are Jenner Investigators and James Martin Senior Fellows. FGH is a former staff member of the Wellcome Trust. The views expressed are those of the authors and do not necessarily represent the views, position or policies of their respective institutions or the NHS, NIHR, the UK Department of Health, the World Health Organization or European Union.

## **Contributions**

This manuscript was drafted by Thomas C. Darton and Christoph J. Blohmke, who both equally contributed. All other authors have provided invaluable input to the quality of this manuscript.

## **Conflict of Interest:**

AJP reports grants from Wellcome Trust, grants from Jenner Institute, grants from NIHR Oxford Biomedical Research Centre, grants from British Society for Immunology, during the conduct of the study.

FGH reports serving as consultant on respiratory virus vaccines (GSK), consultant on respiratory virus diagnostics (Hologic), member of DSMB for influenza vaccine study (Sanofi-Pasteur), and member of DSMB for RSV antiviral trials (Gilead) with honoraria paid to University of Virginia. In 2011-13 both the University of Virginia and he personally received compensation from legal firms/insurance companies for his time in reviewing one patent case regarding zanamivir (Biota/GSK) and medicolegal cases involving fatal influenza and oseltamivir (Roche). He has been an unpaid consultant to multiple companies engaged in developing and/or marketing respiratory virus antivirals, other therapies, and vaccines.

All other authors declare no conflict of interest.

## **Box 1: Future opportunities and major advances arising through the use of human challenge studies**

### **Major Advances**

- Assessment of vaccines in more than 10 challenge models to select those most promising to take forward into field trials.
- Insight into the pathogenesis of infections and testing of new treatments.
- Development of ethical and regulatory frameworks to use human challenge models to improve health through research using *the* relevant model system for human health.

### **Future Opportunities**

- To develop new human challenge models and capacity in existing models to accelerate development of new vaccines and therapeutics.
- To apply new technologies, such as RNAseq to investigate the natural and vaccine-induced resistance to infection.
- To adapt models for the use of genetically modified organism (GMO) in order to directly assess putative virulence factors/antigens for vaccine development.
- To develop challenge strain libraries or repositories and to foster greater harmonisation of shared protocols and development of model frameworks.
- To develop models in relevant populations such as in endemic areas who may have markedly different and more relevant responses to infection due to genetic background/previous exposure/differing microbiome etc.
- To seize the potential of collaborative work between regulatory agencies and to develop a multinational ethical framework for conduct of challenge studies.

**Figure 1: Use and development stage of some of the currently active challenge models.** Several models are currently in different stages of development and use. Most models also investigate infection pathogenesis mechanisms and host immune responses including serological, cellular and molecular responses. Challenge models depicted are described in more detail in the text and accompanying tables.

- 1) ‘Dose Escalation’ – studies aiming to identify the challenge doses and resultant attack rates, required to adequately power subsequent vaccine/therapeutic efficacy studies.
- 2) ‘Vaccine Study’ – direct assessment of vaccine efficacy by administration of challenge at some interval after vaccination has been performed.
- 3) ‘Therapeutic Intervention’ – using challenge as a direct therapeutic approach or to testing the efficacy of a treatment after infection has been caused by challenge.
- 4) ‘Vaccine Screening’ – use of challenge models to evaluate potential vaccine candidates prior to subsequent development stages.

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