REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01/30/2020	Technical Report	

4. TITLE AND SUBTITLE
Layered and Integrated Medical Intervention Technologies (LIMIT) Concept Phase 1: Science-Informed Rules for Layering of Vaccines and Antibody Medical Countermeasures to Protect Against Biological Threat Agents

5a. CONTRACT NUMBER
W56KGU-18-D-0004-0001

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Hofmeyer, Kimberly A.; Tsang, Tiffany;

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The MITRE Corporation
7515 Colshire Drive
McLean, VA 22102

8. PERFORMING ORGANIZATION REPORT NUMBER
PRS-20-0292

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Defense Threat Reduction Agency, Research and Development Directorate

10. SPONSOR/MONITOR'S ACRONYM(S)
DTRA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
DISTRIBUTION STATEMENT A. Approved for public release: distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This concept paper presents the science-informed justification and outlines a methodology to define layering rules for vaccines and antibody MCM and considers how layering can widen the protection window against biological threats. This initial concept build was scoped to and considers preparedness specifically through the administration of MCM during operational planning and predeployment phases to optimize Warfighter protection against biological threat agents during deployment.

15. SUBJECT TERMS
Clinical Medicine, Military Operations (General); integrated medical intervention, layering vaccines, biological threat agents, chemical and biological defense, biodefense

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**		39	Susan Carpenito
					19b. TELEPHONE NUMBER *(Include area code)* 781-271-7646

MTR190669
MITRE TECHNICAL REPORT

MITRE

Layered and Integrated Medical Intervention Technologies (LIMIT) Concept Phase 1

Science-Informed Rules for Layering of Vaccines and Antibody Medical Countermeasures to Protect Against Biological Threat Agents

Author(s): Kimberly A. Hofmeyer, Ph.D.
Tiffany Tsang, Ph.D.

30 January 2020

Defense Threat Reduction Agency,
Research and Development Directorate,
Chemical and Biological Department Dept. No.: Z610
Contract No.: W56KGU-18-D-0004-0001
Project No.: D270MC

McLean, VA

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1 Introduction

The mission of the Warfighter Integration Division (CBW) of the Defense Threat Reduction Agency (DTRA) Chemical and Biological Department is to foster strategic partnerships with chemical and biological science and technology (S&T) communities to deliver the best innovations to Department of Defense (DoD) operational capability and enhance global collaboration in biodefense and global health. This is accomplished through active engagement and S&T portfolio integration with the warfighter via experimentation and concept development, tabletop exercises, war gaming, advanced technology demonstrations, and Warfighter utility assessments.

The DoD Chemical Biological Defense Program Planning Guidance for Fiscal Year 2019-2029 included the objective to field integrated layered chemical, biological, radiological, and nuclear (CBRN) defense capabilities that advance the DoD ability to operate and succeed in a CBRN threat environment. CBW is working toward addressing this guidance through pursuit of a concept to develop a science-informed methodology for layering of medical countermeasures (MCM).

This concept paper presents the science-informed justification and outlines a methodology to define layering rules for vaccines and antibody MCM and considers how layering can widen the protection window against biological threats. This initial concept build was scoped to and considers preparedness specifically through the administration of MCM during operational planning and predeployment phases to optimize Warfighter protection against biological threat agents during deployment.

1.1 Problem Statement and Approach

The 2018 National Defense Strategy lays out a strategic approach to ensure a more lethal and resilient Joint Force in increasingly complex security environments defined by rapid technological change, challenges from adversaries in every operating domain, and the impact on current readiness.[1] The strategy to ensure a more lethal force focuses on several elements, including prioritizing preparedness for war and modernizing key capabilities.

The Multiservice Tactics, Techniques, and Procedures (TTP) for Health Service Support (HSS) in a CBRN Environment notes that medical CBRN defense should be fully integrated into the deliberate planning process to maximize readiness.[2] This includes consideration and use of the tools available to defend against biological threat agents. MCM provide a defensive solution

[1] Summary of the 2018 National Defense Strategy of the United States of America, Washington D.C.: US Department of Defense, 2018.
[2] ATP 4-02.7/MCRP 4-11.1F/NTTP 4-02.7/AFTTP 3-42.3, Multiservice Tactics, Techniques, and Procedures for Health Service Support in a Chemical, Biological, Radiological, and Nuclear Environment, dated 15 March 2016.

against biological threat agents; however, this is limited if a single MCM does not meet all efficacy requirements in a biological threat environment. To address this CBW seeks a concept for layered, integrated MCM defense that is science-based and can be tailored to mission needs.

This initial concept build is scoped to consider biological threat agents, specifically *Bacillus anthracis* and *Zaire ebolavirus* (EBOV); anthrax and Ebola MCM that are in active use or clinical development; and administration of MCM during the predeployment phase of mission planning to maximize protection during deployment. Considering this scope, the primary goal for phase 1 of the Layered and Integrated Medical Intervention Technologies (LIMIT) concept is to develop an approach for layering of vaccines and antibody MCM that expands the window of protection during deployment. The concept lays out the technical reasoning that informs MCM layering rules and how this can be aligned with mission timelines.

2 Alignment of Mission Planning Timelines with MCM Protection Parameters

Mission success in biological threat environments can be critically impacted if a biological threat agent encounter results in symptoms (disease or death) that reduces unit strength beyond a recoverable threshold. Preparedness to reduce or negate vulnerabilities in a biological threat environment ideally focuses on administration of pre-exposure prophylaxis MCM (i.e., vaccines and antibody MCM) during planning/predeployment phases to optimize protection and readiness throughout deployment. This is different from the civilian biodefense approach that largely focuses on post-exposure response planning as population size and lower risk tolerance make pre-exposure prophylactics impractical.

The joint deployment and redeployment processes consists of four phases that are iterative and may occur simultaneously throughout an operation:[3]

- Planning
- Predeployment/pre-redeployment activities
- Movement
- Joint reception, staging, onward movement, and integration

Assessment of the threat environment is one of several operational considerations during planning.[3] Predeployment standards outline the training and unique equipment requirements necessary to prepare supporting personnel and forces for the tactical and environmental conditions, as well as potential or known in theater health threats.[3] Deployment timelines will dictate available time to conduct force preparation activities. Combat deployments may occur within hours or days from receipt of a deployment order while other units may deploy on a timeline of days to several weeks.[3] The Multiservice TTP for HSS in a CBRN Environment notes that medical CBRN defense should be fully integrated into the deliberate planning process to maximize readiness and that appropriate HSS begin simultaneously with the operational planning process to ensure its synchronization with the campaign plan or operation plan.[4] This initial LIMIT concept build considers preparedness specifically through the administration of MCM during operational planning and predeployment phases to optimize Warfighter protection against biological threat agents during deployment.

[3] Joint Publication 3-35, Deployment and Redeployment Operations, Washington D.C.: U.S Department of Defense, January 10, 2018.
[4] ATP 4-02.7/MCRP 4-11.1F/NTTP 4-02.7/AFTTP 3-42.3, Multiservice Tactics, Techniques, and Procedures for Health Service Support in a Chemical, Biological, Radiological, and Nuclear Environment, dated 15 March 2016.

2.1 MCM Protection Parameters

Immunity is the capability of the body to resist biological agents (pathogens), which can cause disease. Vaccines and antibody MCM take advantage of various elements of immunity to mediate protection against pathogens. MCM-mediated immunity can very broadly be categorized as either active or passive. Active immunity results when the body is exposed to a vaccine that induces the body to a produce an immune response itself to mediate protection against the target pathogen. Whereas passive immunity is provided when a specific element of the immune system designed to target a specific pathogen (e.g., antibody) is directly administered to an individual rather than having it be produced by an individual's immune system. MCM that take advantage of active versus passive mechanisms have distinct protection timeline benefits and drawbacks as summarized in Table 2-1.

Table 2-1. Active Versus Passive Immunity.

Immunity Type	MCM Type	Advantage	Disadvantage
Active	Vaccine	**Long-lasting protection**. Vaccines generate immune memory against the specific pathogen that can protect over an extended period.	**Delayed onset of protection**. Vaccines work by active immunity and have to stimulate the body to generate a protective immune response.
Passive	Antibody MCM	**Rapid onset of protection**. Antibody MCM work by passive immunity and are immediately effective once administered.	**Short duration of protection**. Antibody MCM are cleared from the body over several weeks and will no longer provide protection once levels are too low.

The major benefit of vaccines is that they can generate long-lasting protective immune memory responses to a target pathogen. However under tight time constraints the utility of vaccines is limited by the time it to takes to generate a response, which can take weeks, or longer if a booster immunization is required. Conversely antibody MCM can confer rapid protection but do so for significantly shorter durations. Time to and duration of protection must be defined for MCM in order to align with operational timelines. This information should be available to U.S. Government product development managers based on published or unpublished data from MCM developers.

Vaccine protection parameters are often determined through selection of a primary immune surrogate readout that is established as related to how the vaccine mediates protection in humans. The immune surrogate selected is most often focused on detecting pathogen- or antigen-specific antibodies induced by the vaccine but may also be pathogen- or antigen-specific memory T cells. During clinical testing in humans, assays to assess the immune surrogate are conducted at select

timepoints to determine the point at which a protective immune response is achieved (time to protection) and how long the protective immune response is maintained (duration of protection). Duration of protection studies often only extend to a defined point in time (weeks to months) and could be longer than indicated in studies. Protection timelines may also be determined in clinical trials of populations affected by the disease; however, this is less common for traditional biological threat agents as exposure is life threatening and either does not happen in nature (i.e., nefarious use only) or happens in nature rarely and in numbers that are too low to support a standard clinical efficacy trial.

Antibody MCM protection parameters for traditional biological threat agents are often determined through selection of a protective dose in a relevant animal model, commonly primates, and then confirmation of a dose in humans. Animal testing may select a general dose, dose range, or determine a specific concentration of antibody in the blood as protective. Subsequent human testing will assay for the presence and kinetics of antibody in the blood. Antibody administered intravenously will reach its maximum concentration within minutes, so a key goal is to determine how long antibody concentration in the blood remains above a protective level.

2.2 Timeline Alignment Strategy

Vaccines and antibody MCM could be used in combination (i.e., layered) when both a rapid onset and long duration of protection are required. This layering strategy could increase the window of protection that is afforded by either type of MCM alone and could be particularly beneficial in operations with shorter predeployment timelines and longer or undermined deployment timelines. This concept is summarized in Figure 2-1. The top diagram illustrates the desirable trait of vaccines, which is their duration of protection and the undesirable trait, which is the time it takes to reach the threshold of protection. The middle diagram illustrates the desirable trait of antibodies, which is their rapid onset of protection, but short duration. The bottom diagram illustrates the desired effect when these two types of MCM are delivered together, where there is no drop below the threshold of protection and protection is both rapid and of long duration.

A future LIMIT tool would require input of the planning/predeployment and deployment timelines from an operational planner, which would be aligned with protection parameters of MCM (time to/duration of protection) and MCM layering rules to recommend an MCM administration schedule so that the window of protection meets operational timeline needs.

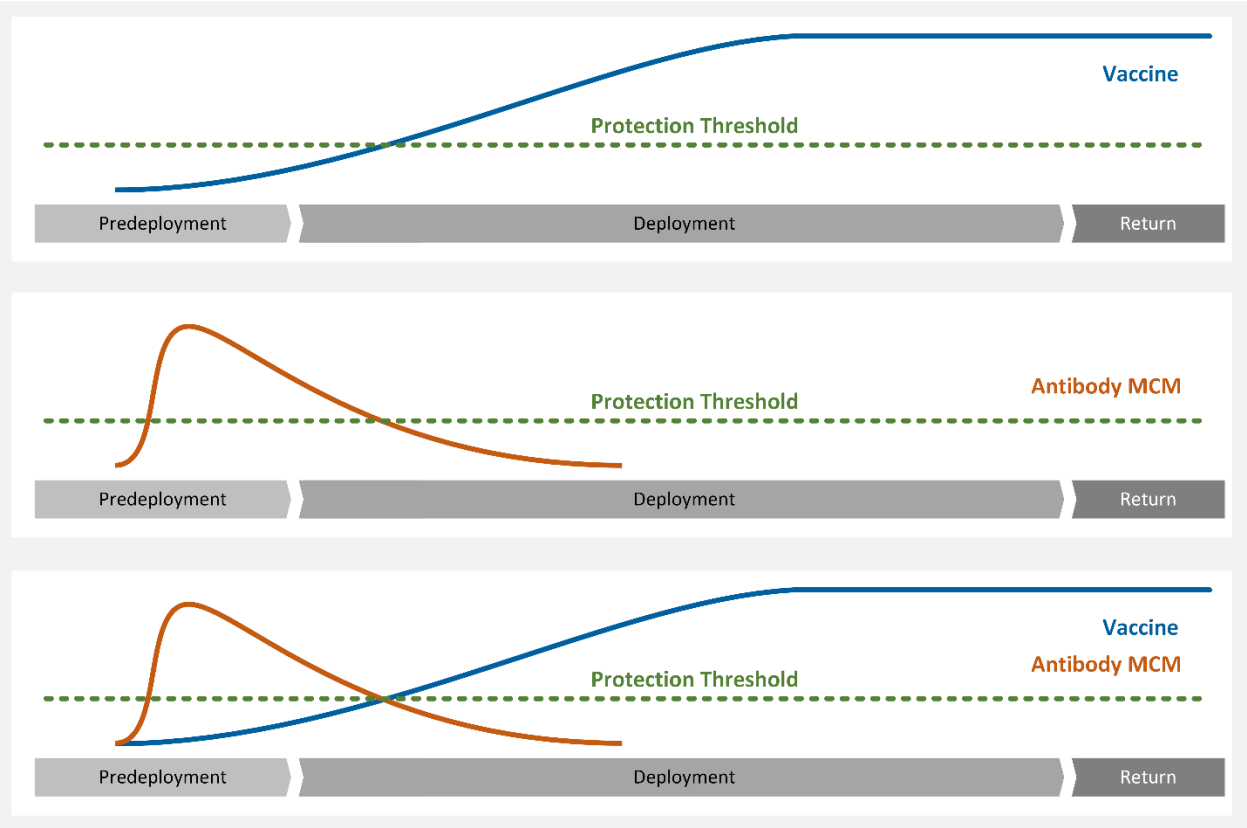

Figure 2-1. Summary of Alignment of MCM Protection and Mission Planning Timelines.

3 MCM Mechanisms of Action

Vaccines and antibody MCM provide protection through active and passive immunity, respectively, as summarized in Table 2-1. At a more defined level, vaccines and antibody MCM mediate protection against pathogens through specific mechanisms. This section presents information on MCM protection mechanisms that could be impacted through layering of vaccines with antibody MCM.

3.1 How Vaccines Generate Protective Immunity

Mechanism of Action. The fundamental concept behind vaccines is to induce an immune response that resembles what is generated against the real pathogen, but without the adverse effects of disease. This vaccine-generated response is then retained as pathogen-specific immunological memory that protects against future pathogen encounter months, even years later.

Rational subunit vaccine design focuses on two key components, called antigen and adjuvant, that play different roles in impacting the immune response. Antigen is a discrete component of a pathogen that the immune response is specifically directed against. Studies in humans or animal models can determine which antigens are targeted by the immune response and result in protection against the pathogen. In vaccine design, known protective antigens are selected to generate the pathogen-specific immune response. Adjuvants stimulate the immune response in a more general sense, which in turn enhances the immune response to the selected antigen. This is summarized in Figure 3-1 and described in more detail below.

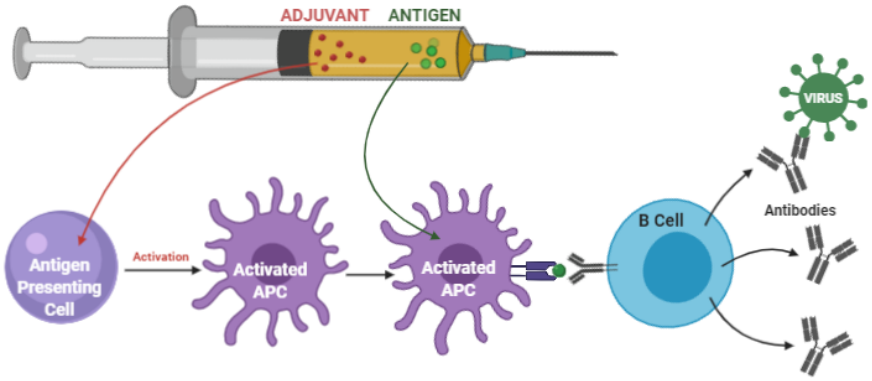

Figure 3-1. Vaccine Components—Adjuvant and Antigen—Mechanism of Action.

Modern adjuvant design is primarily based on developing synthetic versions of specific molecular patterns conserved across classes of pathogens—viruses, bacteria, parasites—that serve as a general warning signal to the immune system that a pathogen is present. These pathogen-associated molecular patterns (PAMPs) are recognized by specific pattern recognition receptors (PRRs) present in immune cells and are a first step in activating an immune response. Different types of PRRs are present in different locations on or within a cell. PRRs that are stimulated by molecular structures on the surface of bacteria or parasites are typically located on

the surface of immune cells. Whereas PRRs that detect viral genomic material are located inside cells since virus propagation depends on internalization and release of genomic material inside cells. Signaling through PRRs is one of the first steps to immune activation and is important to guiding the specific type of immune response that is needed to protect against a specific pathogen.

Another key component of the early immune response generated by a vaccine is the processing and presentation of specific antigens by antigen-presenting cells (APCs). APCs activate other immune cells that can establish long-term pathogen-specific immune memory. Antigen presentation occurs via two distinct pathways that are related to how an APC encounters a pathogen. APCs can engulf (phagocytose) external pathogens or antigen for degradation, processing, and presentation via one pathway. Alternatively, APCs already infected by pathogens can degrade, process, and present antigen via another pathway. Antigens that are processed, and in turn presented, via these two different pathways activate different types of immune memory cells that play different roles in long-term protective immunity.

The immunogenicity of vaccines is critically tied to the ability of the antigen and adjuvant to gain access to the immune cell components responsible for inducing the specific type of immune response that mediates protection against the target pathogen.

Type of Vaccines. Vaccines are designed towards the idea of inducing an immune response similar to that generated by the real target pathogen, while avoiding the effects of disease, and can be broadly categorized into three types:

- Inactivated vaccines are the actual disease-causing pathogen that is killed via heat, irradiation, or chemical methods.
- Live attenuated vaccines are live pathogens weakened via culture or genetic engineering methods that alter virulence genes and result in live organisms that no longer cause disease.
- Subunit vaccines are synthetically designed to contain only select components (or subunits) of pathogens that are minimally necessary to generate a protective immune response.

Whole pathogen inactivated and live attenuated vaccines naturally contain both antigen and adjuvant, while subunit vaccines are strategically designed to contain both components (

Table 3-1).

Table 3-1. Antigen Expression and Adjuvant Sources by Vaccine Type.

Vaccine Type	Antigen Expression	Adjuvant Source
Whole Pathogen Vaccines		
Inactivated vaccine	Naturally present	Contains intrinsic adjuvant structures
Live attenuated vaccine	Naturally present	Contains intrinsic adjuvant structures
Subunit Vaccines		
Protein subunit vaccine	Select pathogen protein antigen(s) either: • Purified from the pathogen • Synthetically produced (recombinant)	Adjuvant must be added separately to vaccine formulation
Viral vectored vaccine (Viral vector backbone from virus that does not cause disease engineered to express antigen of the target pathogen.)	Select pathogen antigen(s) expressed so they are present either: • On the virus surface • Only in virus genomic material	Viral vector backbone contains intrinsic adjuvant structures
Nucleic acid vaccine	Select pathogen antigen(s) expressed as genomic construct, either: • DNA • RNA	Nucleic acid construct may have adjuvant properties itself (self-adjuvating) or adjuvant is added separately

3.2 How Antibody Medical Countermeasures Mediate Protection

Mechanism of action. Protective immunity generated in response to pathogens or vaccines is made up of specific immune cell types that maintain immune memory long-term. At a very high-level immune memory is mediated by T cells and B cells. T cells provide protection by directly killing infected cells in the body or by orchestrating other protective elements of the immune system. B cells produce antibodies specific to the pathogen or antigen, which can mediate protection.

Antibodies are Y-shaped proteins that mediate protection by either directly blocking a pathogen or orchestrating the pathogen-killing functions of other components of the immune system. The V-shaped “arms” of the antibody recognize and bind antigens of a specific pathogen. Antibodies can block or neutralize a pathogen by binding to antigens that are essential for infecting cells or antigens responsible for causing disease. Neutralizing antibodies used against pathogens are typically designed specifically with this mechanism in mind—physically blocking a pathogen

from infecting a cell in the first place or preventing pathogen-derived toxins from harming cells (Figure 3-2).

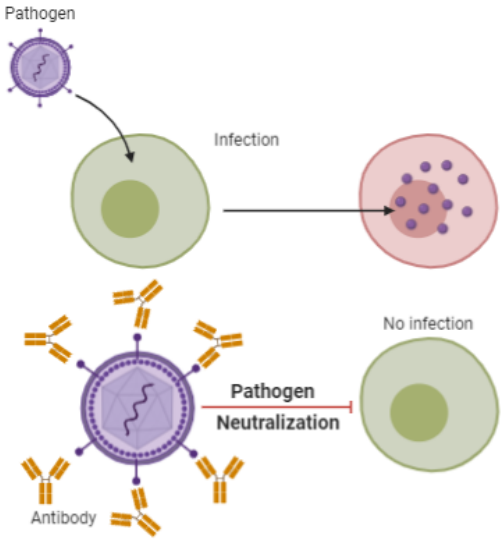

Figure 3-2. Antibody Mechanisms of Action—Neutralization.

In addition to this neutralizing function, the "tail" portion of the antibody structure can flag the pathogen as a foreign agent for destruction by different components of the immune system. Antibody-mediated destruction of pathogens occurs in two main ways (Figure 3-3):

- Cell lysis is the breaking down of the cell wall that results in cell death. Antibodies do not directly cause lysis, but rather recruit other immune system components that then lyse the "flagged" pathogen or pathogen-infected cell.
- Opsonization is the antibody-mediated engulfing (or phagocytosis) of pathogens by immune cells, often APCs, for degradation.

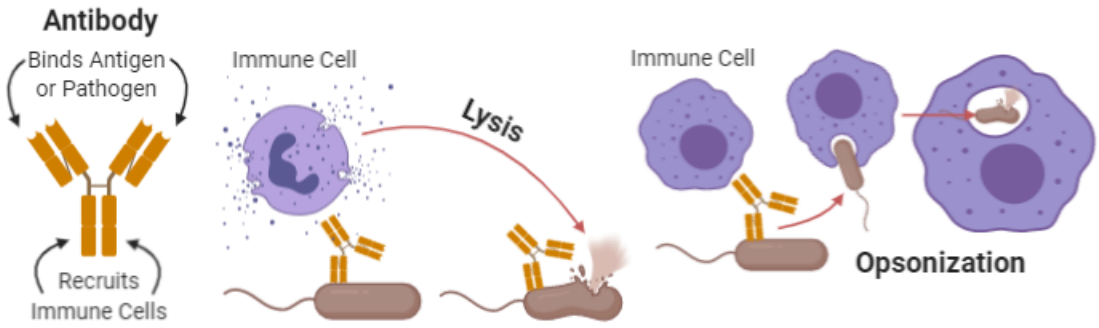

Figure 3-3. Antibody Mechanisms of Action—Lysis and Opsonization.

Types of Antibody MCM. During a normal immune response to a pathogen, antibodies are generated against multiple different pathogen proteins and different parts of individual proteins; this is called a polyclonal antibody response. Antibody MCM may contain antibodies that

recognize multiple different antigens (polyclonal) or antibodies that recognize a single, specific antigen (monoclonal).

Intravenous Immunoglobulin (IVIG) is a blood product typically sourced from individuals that have confirmed exposure to a specific pathogen or vaccine. Plasma or serum, the component of blood that contains antibodies, is isolated from such individuals and pooled for therapeutic use as it contains polyclonal antibodies against the intended pathogen target. Because IVIG contains polyclonal antibodies against the intended pathogen target, it may block multiple biological functions of a pathogen and orchestrate protection via multiple mechanisms.

Monoclonal antibodies are designed against a specific antigen target that neutralizes a key mechanism of disease of the target pathogen. Monoclonal antibody design frequently targets the key protein(s) on the surface of a pathogen critical for binding and infecting host cells, and in turn inhibits infection of cells. Another common target for monoclonal antibodies is any disease-causing, pathogen-produced toxin that is then blocked from killing the host cells. Different monoclonal antibodies targeting different pathogen proteins, or parts of proteins, can be combined into a monoclonal antibody cocktail.

4 Avoiding Negative Outcomes in MCM Layering

The primary goal of the phase 1 LIMIT concept is to expand the window of protection against biological threat agents through layering MCM. However for MCM layering to be effective, interference between MCM must be avoided. Interference could lead to a synergistic negative effect that causes serious immune-mediated damage of host tissues or it could lead to an overall reduction in immunity below that offered by a single MCM. For layering of vaccines and antibody MCM that protect against the same pathogen, interference is possible if there is a shared antigen target.

4.1 Immune-Mediated Damage

Hypersensitivity describes when an immune response occurs in an exaggerated and inappropriate way that causes harm to an individual (immunopathology). There are four types hypersensitivity reactions formally described, one of which could hypothetically occur when vaccines and antibody MCM are coadministered. Type III Hypersensitivity (or immune complex-mediated hypersensitivity) occurs when antibody-antigen complexes (or immune complexes) accumulate in large quantities and are not adequately cleared by the immune system, which leads to accumulation within the vessels or other tissues of patients. Immunopathology occurs when antibody-mediated lysis is triggered and damages the host tissue where the immune complexes are deposited. Type III Hypersensitivity can occur specifically when there is soluble antigen present along with antibodies; thus, negative reactions are hypothetically possible when vaccines are coadministered with antibody MCM in the following scenarios:

- Live viral vaccines (whole virus attenuated or vectored) if virus infection of cells results in the production and release of soluble antigen in excessive quantities, and if the antigen is a target of the coadministered antibody MCM.

- Nucleic acid vaccines if the antigen produced by cells results in release of soluble antigen in excessive quantities, and if the antigen is a target of the coadministered antibody MCM.

- Subunit protein vaccines are usually formulated so the protein antigen remains associated with the adjuvant in the final vaccine formulation; however, some antigen can remain free. If an adjuvanted subunit protein vaccine has excessive quantities of soluble antigen or if the vaccine is unadjuvanted and contains only soluble antigen, a reaction could hypothetically occur if the antigen is a target of the coadministered antibody MCM.

4.2 Interference with MCM-Mediated Protection

4.2.1 Vaccine Impact on Antibody-Mediated Protection

Vaccines most likely have limited impact on protection mediated by antibody MCM. However, if a vaccine and antibody MCM share an antigen, the antibody-mediated protection could hypothetically be decreased if the vaccine antigen is bound by the antibody. If antibody MCM binding to the vaccine antigen decreases the free antibody available to a level below the effective therapeutic amount, protection mediated by the remaining free antibody MCM may be insufficient if the real target pathogen is encountered. However, antibody MCM are typically administered at high doses that are unlikely to be overwhelmed by the amount of vaccine. While hypothetically possible coadministration of vaccines and antibody MCM with a shared antigen target have not shown any negative impact on antibody MCM protection.[5,6] Negative impacts of vaccines on therapeutic antibodies will not be considered as a source of interference in MCM layering.

4.2.2 Antibody MCM Impact on Vaccine-Mediated Protection

Antibody MCM are typically designed to neutralize a pathogen by binding to a molecule essential for pathogen survival (i.e., infection) or causing disease. Vaccine immunogenicity could be inhibited if the vaccine and antibody MCM share an antigen and the vaccine antigen is in a form that can be bound by the antibody MCM. This is particularly true if the vaccine antigen mediates a process, for example infection of a target cell, which is critical to the mechanism of how it generates immunity.

The possible impacts of antibody MCM on vaccine immunogenicity can be considered from the perspective of how preexisting immunity—from either natural pathogen exposure or maternally acquired antibody—impacts vaccine immunogenicity. Preexisting immunity in neonates is acquired through placental transfer of maternal antibodies present in the mother from previous infection or vaccination. The presence of preexisting neutralizing antibodies targeting vaccine vectors can reduce vaccine immunogenicity by preventing the vaccine from accessing (i.e., infecting) cells or by reducing the contact time between B cells and vaccine-encoded antigens.[7] Inhibition of vaccine immunogenicity by passively acquired maternal antibody matched to the vaccine is demonstrated in humans with several vaccine types including whole cell inactivated, live attenuated vaccines, and subunit protein vaccines.[8] Masking of vaccine antigen and the

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[5] JE Eyles *et al*. "Concomitant administration of Yersinia pestis specific monoclonal antibodies with plague vaccine has a detrimental effect on vaccine mediated immunity." *Vaccine* 25 (2007).
[6] NV Malkevich *et al*. "Effect of anthrax immune globulin on response to BioThrax (anthrax vaccine adsorbed) in New Zealand white rabbits." *Antimicrob Agents Chemother* 57 (2013).
[7] M Manvendra *et al*. "Pre-existing immunity against vaccine vectors – friends or foes?" *Microbiology* 159 (2013).
[8] S Niewiesk. "Maternal Antibodies: Clinical Significance, Mechanism of Interference with Immune Responses, and Possible Vaccination Strategies" *Frontiers in Immunology* 16 (2014).

inhibitory effect on B cell memory response has been observed for vaccination in the presence of maternally acquired antibodies and for influenza vaccination in the presence of preexisting antibodies from previous infection of vaccination.[8,9]

The LIMIT concept is primarily concerned with avoiding any interference and negative impacts that might occur between layered MCM as described above. However, pre-existing antibody immunity can in some instances have a positive effect on vaccine immunogenicity. Formation of antigen:antibody immune complexes can result in a type III hypersensitivity reaction, but this may specifically be in the context of complexes where the antibody has low affinity for the antigen. Immune complexes where the antibody has sufficiently high affinity for antigen can enhance APC interaction with antigen, inducing a high titer antibody and T cell response.[10] Preexisting antibodies to influenza increase immunogenicity of influenza virosome-based vaccines. Influenza virosomes are synthetically assembled particles constructed to resemble the influenza virus, but without genomic material, and containing an antigen of choice; preexisting influenza antibodies are hypothesized to improve immunogenicity by improving uptake of vaccine by APCs.[11] While it may hypothetically be possible for antibody MCM to improve vaccine administration when coadministered, the conservative approach is to focus on avoiding negative interactions.

[9] MR Castrucci. "Factors affecting immune responses to the influenza vaccine." *Human Vaccines & Immunotherapeutics* 14 (2017).
[10] X Wang *et al*. "From therapeutic antibodies to immune complex vaccines." *npj Vaccines* 4 (2019).
[11] C Moser *et al.* "Influenza virosomes as a combined vaccine carrier and adjuvant system for prophylactic and therapeutic immunizations." *Expert Review of Vaccines* 6 (2007).

5 MCM Layering Rules

MCM layering rules may be considered when a lack of interference between MCM has not been empirically tested. Some combinations of *B. anthracis* MCM have been tested and data publicly released on whether the specific combination is okay and does not interfere with individual MCM-mediated protection. Approved, or select advanced candidate, vaccines and antibody MCM combinations for *B. anthracis* are summarized in Figure 5-1 and discussed further, along with other tested combinations, in Section 5.2. While empirical testing for combinations has not been conducted, MCM layering rules may inform layering decisions.

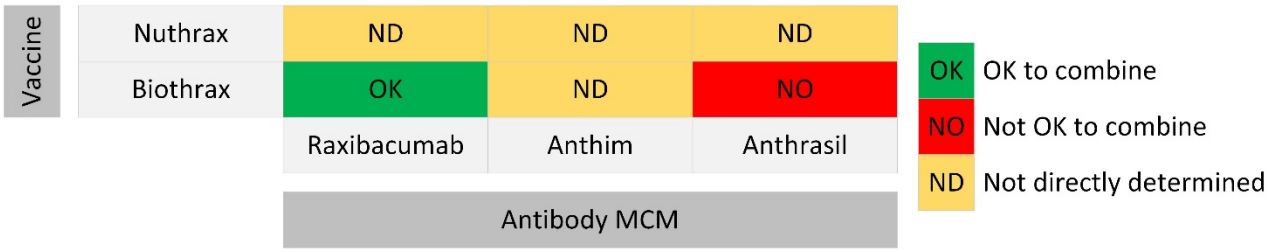

Figure 5-1. *B. anthracis* Vaccines and Antibody MCM Combinations.

MCM layering rules serve two key purposes: rational MCM design and layering of existing MCM. Rational MCM design would consider the MCM types available or in development and then intentionally design complementary MCM to avoid interference and enable layering. This could be extended to rational design of overall MCM development programs including setting how MCM layering could inform science-based requirements. Layering rules could also be applied as a rule of thumb to inform medical decisions for potential layering of approved MCM.

Intuitive immunology-based layering rules were developed to avoid MCM interference. The high-level MCM characteristics that inform rules along with the rules themselves are discussed in Section 5.1. A literature review for instances where antibody MCM and vaccines were coadministered was conducted and presented below.

5.1 Vaccine and Antibody MCM Layering Rules

5.1.1 High-Level MCM Characteristics to Inform Layering Rules

The key characteristics of vaccines and antibody MCM to inform layering rules should be defined as high level as is feasible. This is important so that layering rules can be broadly applied across multiple types of vaccine types in a pathogen agnostic manner. Simplified layering characteristics and rules would also make coding a LIMIT tool in the future more achievable. The fundamental layering consideration for vaccines and antibody MCM is to avoid negative impact on the ability of vaccines to generate protective immune memory. Antibody MCM may be able to interfere with vaccine immunogenicity if an antigen is shared and the vaccine antigen can be bound by the antibody MCM and interrupt generation of vaccine-mediated immunity. With this in mind the high-level characteristic for vaccines is their antigen and how that antigen is expressed; an overview of how different vaccine types express antigen is in

Table 3-1.

The key high-level characteristic of antibody MCM is their antigen target, as antibody binding to vaccine antigen can prevent the vaccine from accessing components of the immune response critical to how it generates immunity. Whether a vaccine antigen is accessible to be bound by an antibody MCM is based on how the antigen is expressed. When a vaccine antigen is expressed exclusively within a vaccine platform (e.g., as part of the genome of a viral vector vaccine) or as an independent nucleic acid-based construct, it cannot be bound by an antibody MCM (i.e., not accessible) even if the antigen is common between the MCM types. This concept is summarized in Figure 5-2 where in both scenarios the vaccines and antibody MCM share a target antigen (represented in blue); however, in the first scenario the antigen is only encoded in DNA whereas in the second the antigen is expressed as a surface protein where it can be bound by the antibody MCM and disrupt an early step the process of generating the vaccine immune response.

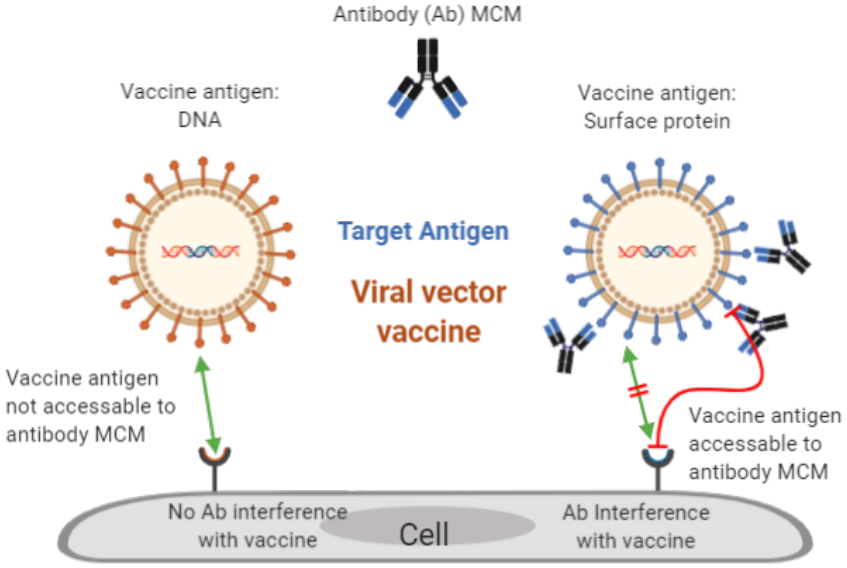

Figure 5-2. Vaccine and Antibody MCM Layering Interference Based on Vaccine Antigen Expression.

An overview of the key characteristics of select anthrax (*Bacillus anthracis*) and Ebola (*Zaire ebolavirus*) vaccines and are described in Table 5-1 and Table 5-2, respectively.

Table 5-1. Key Vaccine Characteristics – Select Anthrax and Ebola Vaccines.

Pathogen	Vaccine Name	Vaccine Antigen		
		Target	Expression	Accessible
Anthrax	Biothrax	PA (LF, EF)	Purified protein	Yes
	NasoShield	PA	Viral vector DNA	No
Ebola virus	INO-4212	GP	Plasmid DNA	No
	cAd3-EBO Z	GP	Viral vector DNA	No
	Ad26.ZEBOV	GP	Viral vector DNA	No
	MVA-BN Filo	GP	Viral vector surface	Yes
	rVSV-ZEBOV	GP	Viral vector surface	Yes
	HPIV3-EboZ GP	GP	Viral vector surface	Yes
	EBOV GP	GP	Recombinant protein	Yes

(PA = protective antigen; LF = lethal factor; and EF = Edema factor are components of anthrax toxin; GP = glycoprotein; the name of the surface protein of EBOV that mediates how the virus infects cells; Viral vector vaccines: Ad = adenovirus; MVA = modified vaccinia Ankara; HPIV = human parainfluenza virus)

Table 5-2. Key Antibody MCM Characteristics – Select Anthrax and Ebola Vaccines.

Pathogen	Antibody MCM Name	Antigen Target
Bacillus anthracis	Obiltoxaximab	PA
	Anthrasil	PA (LF, EF)
Zaire ebolavirus	Zmapp	GP
	REGN-EB3	GP
	mAb114	GP

5.1.2 MCM Layering Rules

The primary goal for initial LIMIT concept development is to define simple rules to layer vaccines and antibody MCM to extend the window of protection afforded by either MCM type alone, while avoiding negative interference with how the individual MCM mediate protection; this concept is summarized in Figure 5-3.

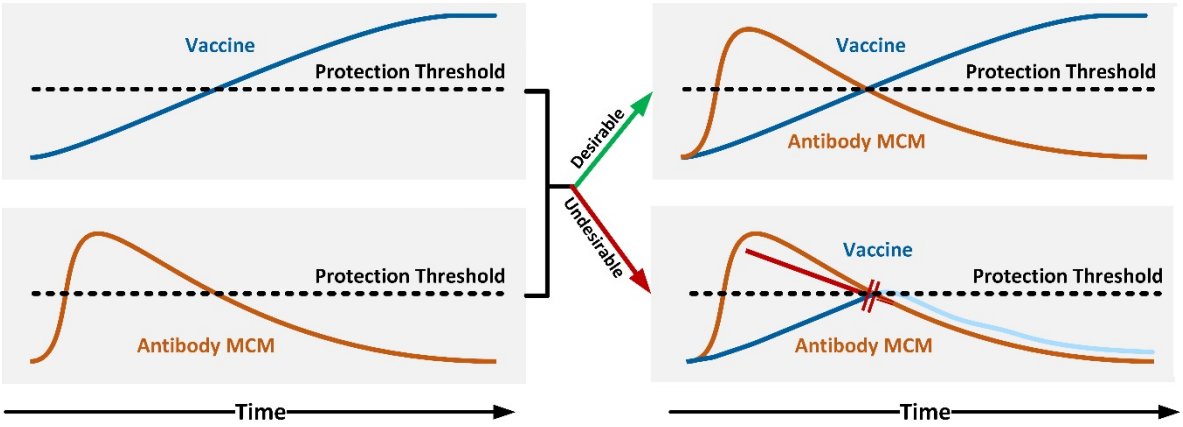

Figure 5-3. Summary of the Goal of MCM Layering.

The focus of layering rules for vaccines and antibody MCM is to increase the likelihood that an antibody MCM administered along with a vaccine will not interfere with any processes critical to how the vaccine generates protective immunity. The immunogenicity of vaccines is critically tied to its key components—antigen and adjuvant—gaining access to the immune cell components responsible for inducing the specific type of immune response that mediates protection against the target pathogen. Antibody MCM are typically designed against a specific antigen target that neutralizes a key biological function of the target pathogen critical to how it causes disease. If the antibody is able to bind vaccine antigen, it could negatively impact immunogenicity. MCM layering rules to avoid this are summarized in Figure 5-4. **Layering Rules for Vaccines and Antibody MCM.**

4.

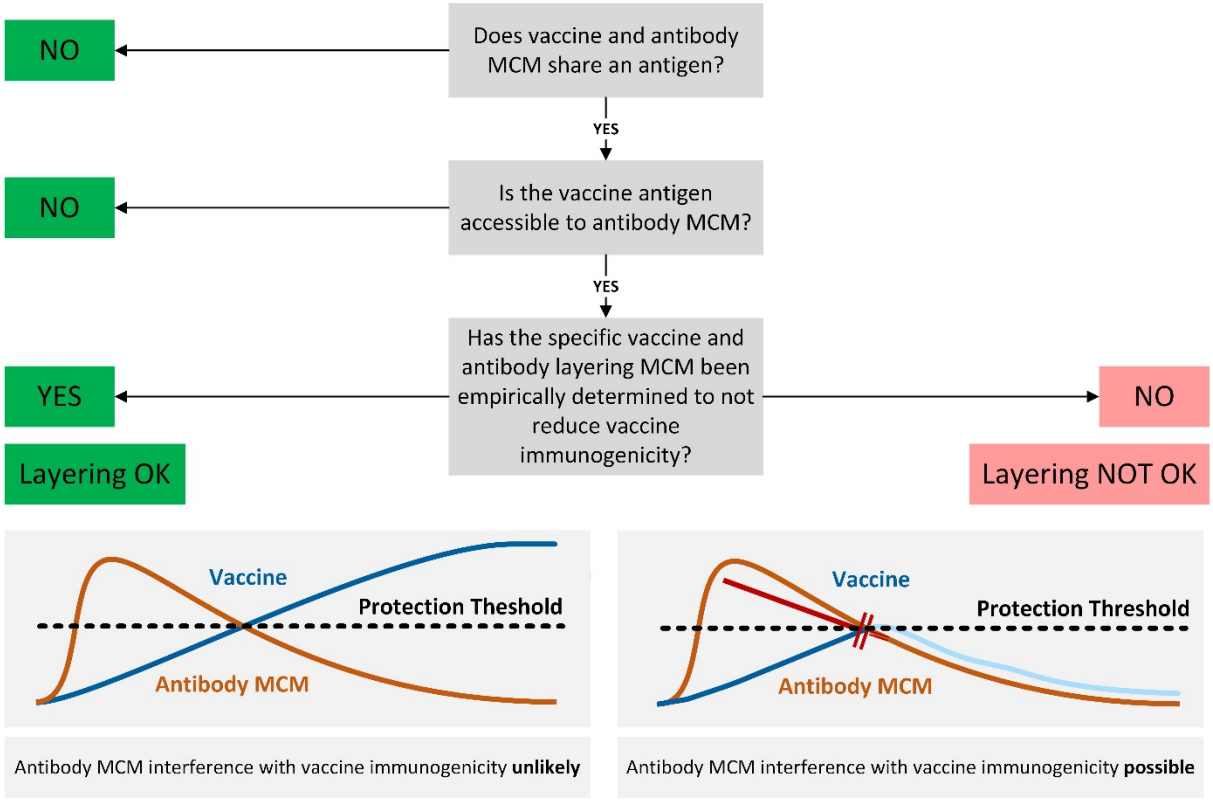

Figure 5-4. Layering Rules for Vaccines and Antibody MCM.

This decision tree was used to evaluate the types of Ebola vaccines presented in Table 5-1 for hypothetical use in combination with mAb114, which all share the same antigen target. While mAb114 shares an antigen (GP) with the DNA and adenovirus vector Ebola vaccines, layering is possible because mAb114 cannot bind the GP vaccine antigen as it is either not in a form recognizable to the antibody MCM (DNA and Ad) or is expressed exclusively within the viral vector (Ad). mAb114 is not recommended for layering with the remaining Ebola vaccine types (MVA, VSV, HPIV3, recombinant protein nanoparticle) as the vaccine antigen is expressed in a form recognized by the antigen (protein) and is accessible to mAb114 binding. The results are summarized in Table 5-3.

Table 5-3. Combination of mAb114 with Vaccine Platform Expressing the Same Antigen – Hypothetical Outcomes.

Ab MCM	**Vaccine Platform Expressing Ebola GP**					
	DNA	Ad	MVA	VSV	HPIV3	Recombinant protein Nanoparticle
mAb114	OK	OK	NO	NO	NO	NO

(NO = Not OK; Viral vector vaccines: Ad = adenovirus; MVA = modified vaccinia Ankara; HPIV = human parainfluenza virus, VSV= vesicular stomatitis virus)

5.2 Literature Review of Vaccine and Antibody MCM Layering

A literature review was conducted to find instances where vaccines and antibody MCM targeting the same pathogen were coadministered and are summarized in Table 5-4. Representative research publications are cited for instances where there is an abundance of publications presenting the same outcomes. Search terms focused on variations of active and passive immunization; combined vaccine and antibody; coadministration of vaccine and antibody; and concomitant administration. The literature search was thorough but is not necessarily exhaustive. Table 5-4 summarizes the following information: target pathogen; type of antibody MCM (IVIG or monoclonal antibody [mAb]) and target; type of vaccine; vaccine antigen (ag) characteristics; impact on vaccine immunogenicity; and references.

Table 5-4. Literature Review Summary – Layered Vaccines and Antibody MCM.

Target Pathogen	Antibody MCM		Vaccine				Out come	Notes
			Type	Antigen				
	Ag	Type		Target	Expression	Access.		
Maternal Hepatitis B virus	Whole virus	IVIG	Recombinant HepB vaccine	HepB surface antigen	Recombinant protein	Yes	OK	Boosted after coadmin[12]
Maternal Hepatitis B virus	Whole virus	IVIG	Inactivated whole virus	Whole virus	whole virus	Yes	OK	Boosted after coadmin[13]
Maternal HIV-1	gp120	mAb	Recombinant gp120 protein (rgp120) + 3M052-SE	gp120	Recombinant protein	Yes	OK	Also tested rgp120+3M052 -SE plus MVA-gp120; but no MVA alone control[14]
Bacillus anthracis	PA	IVIG	Purified protein + Alum	PA	Purified protein	Yes	NO	IVIG from Anthrax Vaccine Adsorbed (AVA)-vaccines[15]

[12] H Zou *et al*, "Protective Effect of Hepatitis B Vaccine Combined with Two-Dose Hepatitis B Immunoglobulin on Infants Born to HBsAg-Positive Mothers" *PloS ONE* 6 (2011).
[13] H Tada *et al*, "Combined passive and active immunization for preventing perinatal transmission of hepatitis B virus carrier state." *Pediatrics* 70 (1982).
[14] M Dennis *et al*, "Coadministration of CH31 Broadly Neutralizing Antibody Does Not Affect Development of Vaccine-Induced Anti-HIV-1 Envelope Antibody Responses in Infant Rhesus Macaques" *Journal of Virology* 93 (2019).
[15] NV Malkevich *et al*, "Effect of Anthrax Immune Globulin on Response to BioThrax (Anthrax Vaccine Adsorbed) in New Zealand White Rabbits" *Antimicrobial Agents and Chemotherapy* 57 (2013).

Bacillus anthracis	PA	mAb	Purified protein + Alum	PA	Purified protein	Yes	OK	Raxibacumab tested[16]
Measles virus	Whole virus	IVIG	Live, attenuated virus	Whole virus	Whole virus	Yes	NO	[17]
Measles virus	Whole virus	IVIG	Vaccinia vector-HA	HA	Viral vector surface	Yes	NO	[6]
Measles virus	Whole virus	IVIG	Recombinant protein HA + ISCOMs	HA	Recombinant protein	Yes	OK	[6]
Hepatitis A virus	Whole virus	IVIG	Inactivated whole virus	Whole virus	Whole virus	Yes	NO	Boost able to overcome impact[18]
Respiratory syncytial virus	Whole virus	IVIG	Recombinant fusion and large GPs + Alum	Surface GPs	Recombinant protein	Yes	NO	Rats: reduced Ab and protection[19]
Polio virus	Whole virus	IVIG	Live, attenuated oral polio vaccine	Whole virus	Whole virus	Yes	OK	[20,21]
Rabies virus	Whole virus	IVIG	Inactivated whole virus	Whole virus	Whole virus	Yes	NO	Impacted by human IVIG, less by equine IVIG[22]
Rabies virus	GP and nucleoprotein	mAb cocktail	Inactivated whole virus	Whole virus	Whole virus	Yes	NO	Mice: reduced Ab but not T cells[23]
Tetanus toxin	Toxin	IVIG	Td-pur, toxin + Alum	Toxin	Protein	Yes	OK	[24]

[16] W Bower, "ACIP Anthrax Vaccine Work Group" Presentation, Advisory Committee on Immunization Practices Working Group, Silver Spring, MD, October 23 2018.
[17] RS van Binnendijk *et al*, "Protective immunity in macaques vaccinated with live attenuated, recombinant, and subunit measles vaccines in the presence of passively acquired antibodies." *Journal of Infectious Diseases* 175 (1997).
[18] A Zanetti *et al*, "Does immunoglobulin interfere with the immunogenicity to Pasteur Mérieux inactivated hepatitis A vaccine?" *Journal of Hepatology* 26 (1997).
[19] BR Murphy *et al*, "Effect of passive antibody on the immune response of cotton rats to purified F and G glycoproteins of respiratory syncytial virus (RSV)" *Vaccine* 9 (1991).
[20] JE Kaplan *et al*, "The effect of immune globulin on the response to trivalent oral poliovirus and yellow fever vaccinations" *Bulletin of the World Health Organization* 62 (1984).
[21] MS Green *et al*, "Response to trivalent oral poliovirus vaccine with and without immune serum globulin in young adults in Israel in 1988." *Journal of Infectious Disease* 162 (1990).
[22] J Lang *et al*, "Suppressant effect of human or equine rabies immunoglobulins on the immunogenicity of post-exposure rabies vaccination under the 2-1-1 regimen: a field trial in Indonesia. MAS054 Clinical Investigator Group" *Bulletin of the World Health Organization* 76 (1998).
[23] CL Schumacher *et al*, "Inhibition of immune responses against rabies virus by monoclonal antibodies directed against rabies virus antigens." *Vaccine* 10 (1992).
[24] J Shin *et al*, "Influences on Formation of Tetanus Antibody after Simultaneous Injection of Tetanus Immunoglobulin with Tetanus Vaccine" *Journal of Korean Medical Science* 27 (2012).

Yersinia pestis	LcrV and F1	mAb	Recombinant LcrV and F1 + Alum	LcrV and F1	Recombinant protein	Yes	NO	Mice: pre-exposure MCM[25]
Tick-borne encephalitis virus	IVIG	IVIG	Inactivated whole virus	Whole virus	Whole virus	Yes	NO	Human: reduced Ab, after boost OK [26] Mouse: reduced protection[27]

The proposed layering rules posit that coadministration of antibody MCM and vaccines that share the same antigen target can be layered if the vaccine antigen is expressed in a way that cannot be bound by the antibody; for example, adenovirus vaccine vectors express target antigen only in viral DNA. However, literature review did not return publications with this type of combination.

All literature identified involved layering of antibody MCM that shared and could hypothetically bind vaccine antigen. For combinations of IVIG and vaccines targeting the same pathogen, a clear pattern did not emerge in the literature review based on vaccine type or how vaccine antigen is expressed. Instances where monoclonal antibody was coadministered with vaccines was limited but observed in four instances. The layering of an inactivated whole rabies virus vaccine with a monoclonal antibody cocktail against rabies inhibited vaccine immunogenicity as did layering of an adjuvanted recombinant protein vaccine for *Y pestis*.[28] In the two other publications adjuvanted recombinant protein vaccines were layered with monoclonal antibodies that recognize the vaccine antigen and did not have any negative impact on vaccine immunogenicity.[29,30] For both IVIG and mAb, the instances where vaccine immunogenicity was reduced it was reported several times that antibody responses were reduced but T cell responses were not impacted. This may be due to an immunological mechanism that specifically impacts B cells. For example, antibody MCM-mediated sequestration of vaccine antigen, which may prevent B cell recognition of antigen and in turn reduces generation of a memory B cell response and antibodies.

[25] JE Eyles *et al*, "Concomitant administration of Yersinia pestis specific monoclonal antibodies with plague vaccine has a detrimental effect on vaccine mediated immunity" *Vaccine* 25 (2007).
[26] M von Hedenstrom *et al* "Vaccination against tick-borne encephalitis (TBE): influence of simultaneous application of TBE immunoglobulin on seroconversion and rate of adverse events." *Vaccine* 13 (1995).
[27] TR Kreil *et al*, "Passive immunization reduces immunity that results from simultaneous active immunization against tick-borne encephalitis virus in a mouse model" *Vaccine* 16 (1998).
[28] CL Schumacher *et al*, "Inhibition of immune responses against rabies virus by monoclonal antibodies directed against rabies virus antigens." *Vaccine* 10 (1992).
[29] M Dennis et al, "Coadministration of CH31 Broadly Neutralizing Antibody Does Not Affect Development of Vaccine-Induced Anti-HIV-1 Envelope Antibody Responses in Infant Rhesus Macaques" Journal of Virology 93 (2019).
[30] W Bower, "ACIP Anthrax Vaccine Work Group" Presentation, Advisory Committee on Immunization Practices Working Group, Silver Spring, MD, October 23 2018.

Literature review indicates that despite intuitive immunology informed layering rules, there may be instances where layering of vaccines and antibody MCM against the rules are acceptable. However, layering of antibody MCM and vaccines targeting the same pathogen, when the vaccine antigen is expressed in a manner that can be bound by antibody, should be avoided unless a specific combination is empirically determined to not impact vaccine immunogenicity.

6 Case Studies

Cases studies are presented to look at different mission timelines and how MCM layering during the pre-deployment phase could expand the window of protection afforded by any MCM alone. Case studies present five repeated MCM administration scenarios that include administration of antibody MCM or vaccine alone as well as layering of the antibody MCM with one of the vaccines. The general parameters that are applied across all three case studies are described below; case studies A-C timelines are presented separately.

Mission Timelines. Three hypothetical mission timelines were derived from publicly available information; each timeline will individually be the basis for the specific scenario.[31] The timelines also assume a hypothetical point in time when MCM are administered during predeployment.

Table 6-1. Hypothetical Mission Timelines for Case Studies.

Case Study	Timeline Source	Predeployment	Deployment
A	Operation United Assistance – 101st Airborne.[31] Execute order issued 15 September 14; 101st Airborne arrive October 14; 101st Airborne return March 15	19 days	20 weeks
B	Operation United Assistance – temp enabling forces. Execute order issued 15 September 14; Temporary force arrives 20 September 14; Port Opening operational 28 September 14 (assumption: return soon after)	5 days	14 days
C	Navy Individual Augmentee.[32] Predeployment presented as 120 days No return timeline provided, used hypothetical	120 days	12 months

Threat Agent. Hypothetical exposure to Ebola virus. Mortality rate of untreated Ebola is 50 percent.[33]

[31] Operation UNITED ASSISTANCE: The DoD Response to Ebola in West Africa, Joint and Coalition Operation Analysis (J-7), U.S. Department of Defense, January 6, 2017.
[32] U.S. Department of the Navy, Navy Individual Augmentee, Train & Equip. https://www.public.navy.mil/ia/Pages/TE.aspx?TE
[33] Joint Publication 3-11, Operations Chemical, Biological, Radiological, and Nuclear Environments, U.S. Department of Defense, October 29, 2018.

Medical Countermeasures. Three Ebola MCM were selected for case studies; one antibody (mAb144) and two vaccines (rVSV-ZEBOV and cA3-EBO Z). The hypothetical protection parameters (time to/duration of protection) are based on published data, although some conjecture is involved and explained if used. Protection parameters are presented in the individuals case studies in a simplified manner that assumes defined protection timeline boundaries and does not account for prolonged ramp up or down of protection windows.

Table 6-2. Hypothetical MCM Protection Parameters for Case Studies.

MCM	Protection Parameters		
	Time to	Duration of	Explanation
mAb114 (mAb)	0 days	~30 days	In non-human primates: Cmax of 50mg/kg dose 1349.73μg/mL protective, given up to 5d after lethal EBOV exposure; 30mg/kg dose protective 5d after lethal EBOV infection. In human Ph1 PK study, Cmax of 25mg/kg dose 829.38μg/mL.[34]
rVSV-ZEBOV (VSV)	10 days[35]	6 months	Duration: Antibody titers capable of neutralizing Ebola virus were low and declined within six months when tested in a subset of volunteers receiving either a high dose (10–50 million plaque-forming units [pfu]) or a low dose (300,000 pfu) of the vaccine.[36]
cAd3-EBO Z (cAd3)	4 weeks	12 months	Ebola-specific antibodies were induced after a single immunization in all participants at week four; assessment of the durability of the antibody response showed that titers remained high at week 48.[37]

Information Presentation for Case Studies. The window of protection afforded by individual MCM (Table 6-2) is represented approximately by time period in Table 6-3 through Table 6-5. Shaded cells represent when the protection window occurs for each MCM. The colors represent the protection afforded by each MCM; in cases where two MCMs are administered and are providing protection, the boxes are shaded with the blended color of each MCM (e.g., mAb [yellow] plus VSV [blue] displays as green during weeks when protection is afforded by both). The approximate time of MCM administration is represented with an asterisks (*). Periods of time during deployment that are hypothetically vulnerable to attack are **blacked out**.

[34] MR Gaudinski *et al*, "Safety, tolerability, pharmacokinetics, and immunogenicity of the therapeutic monoclonal antibody mAb114 targeting Ebola virus glycoprotein (VRC 608): an open-label phase 1 study" *The Lancet* 393 (2019).
[35] A Henao-Restrepo et al. "Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!)" The Lancet 389 (2017).
[36] A Huttner *et al*, "Determinants of antibody persistence across doses and continents after single-dose rVSV-ZEBOV vaccination for Ebola virus disease: an observational cohort study." *Lancet Infectious Diseases* 18 (2018).
[37] JE Ledgerwood *et al*, "Chimpanzee Adenovirus Vector Ebola Vaccine." *NEJM* 376 (2017).

6.1 Case Study A (Predeployment 19 Days; Deployment Five Months)

Mission Timeline. Mission timeline parameters are represented approximately by week in Table 6-3.

- Predeployment – 19 days
- Deployment – five months

Table 6-3. Case Study A (Predeployment: 19 Days; Deployment Five Months).

	Pre-D (Weeks)			Deployment (Weeks)																		
	1	2	3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
mAb			*																				
VSV			*																				
cAd3			*																				
mAb + VSV			*																				
mAb + cAd3			*																				

Case Study Outcome. Case Study A presents a scenario with a short predeployment phase that could represent two hypothetical scenarios where MCM administration occurs one week before deployment: (1) it took approximately two weeks for MCM to be requested and arrive on location or (2) forces arrive on location one week before deployment. With this administration timing scenario, no MCM alone provides a protection window that adequately covers the full five-month deployment phase. Due to the time to reach protective immunity, both vaccines alone would leave a window of vulnerability open to attack early in deployment. An Ebola bioincident within the vulnerable time period could result in substantial loss of forces, including an estimated 50 percent mortality and an unknown number out of commission due to severe disease symptoms; operational readiness would be significantly impacted.

Two MCM layering options are presented that provide a protection window that adequately covers the full five-month deployment phase: (1) mAb + VSV or (2) mAb + cAd3. In this hypothetical scenario, layering combination 1 is preferred as there is an overlapping protection window that may offer more complete protection early in deployment.

6.2 Case Study B (Predeployment Five Days; Deployment 14 Days)

Mission Timeline. Mission timeline parameters are represented approximately by day in Table 6-4.

- Predeployment – five days
- Deployment – 14 days

Table 6-4. Case Study B (Predeployment: Five Days; Deployment 14 Days)

	Predeployment (Days)					Deployment (Days)													
	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11	12	13	14
mAb			*																
VSV			*																
cAd3			*																
mAb + VSV			*																
mAb + cAd3			*																

Case Study Outcomes. Case Study B presents a scenario with a very short predeployment phase (< one week) in which MCM are hypothetically available on location; this results in MCM administration three days before deployment.

With this scenario, mAb alone provides a protection window that adequately covers the full 14-day deployment phase. Due to the time to reach protective immunity, both vaccines alone would leave a window of vulnerability open to attack during deployment. An Ebola bioincident within the vulnerable time period could result in substantial loss of forces, including an estimated 50 percent mortality and an unknown number out of commission due to severe disease symptoms; operational readiness would be significantly impacted.

In this scenario, mAb alone would be sufficient to provide protection throughout deployment. However, if there was any uncertainty in the deployment phase layering of mAb + VSV would offer protection if deployment was extended.

6.3 Case Study C (Predeployment: 120 Days; Deployment: 12 Months)

Mission Timeline. Mission timeline parameters are represented approximately by week in Table 6-5.

- Predeployment – 120 days (12 weeks)
- Deployment – 12 months

Table 6-5. Case Study C (Predeployment: 120 Days; Deployment 12 Months).

Scenario			Predeployment (weeks)												Deployment (months)											
			1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
	C1	mAb						*																		
		VSV						*																		
		cAd3						*																		
		mAb + VSV						*																		
		mAb + cAd3						*																		
	C2	mAb												*												
		VSV												*												
		cAd3												*												
		mAb + VSV												*												
		mAb + cAd3												*												

Case Study Outcome. Case Study C presents two scenarios, both with a four-month predeployment phase, but different hypothetical timing in access to and administration of MCM.

In scenario C1, MCM arrive on location within ~five weeks and are administered ~seven weeks before deployment. In this scenario, the VSV vaccine provides protection early but may leave forces vulnerable for the majority of the deployment phase. The VSV vaccine could hypothetically provide protection across the full deployment phase with a booster vaccination; however, forward deployment of a vaccine

may be impractical in austere environments without a cold chain. The cAd3 vaccine would provide protection across the full deployment phase of 12 months. Also administration of mAb that early does not have any added benefit to the overall window of protection.

In scenario C2, MCM arrive on location within ~11 weeks and are administered ~ one week before deployment.

With this administration timing scenario, no MCM alone provides a protection window that adequately covers the full 12-month deployment phase. The VSV vaccine alone provides protection early but not throughout the full deployment phase. The cAd3 vaccine provides protection throughout the majority of the deployment phase, but leaves a short, early window of vulnerability. Two MCM layering options are presented: (1) mAb + VSV or (2) mAb + cAd3.

Layering option 1 (mAb + VSV) still leaves an extended window of vulnerability during the second half of the deployment phase. An Ebola bioincident within the vulnerable time period could result in substantial loss of forces, including an estimated 50 percent mortality and an unknown number out of commission due to severe disease symptoms; operational readiness would be significantly impacted. The VSV vaccine could hypothetically provide protection across the full deployment phase with a booster vaccination; however, forward deployment of vaccine may be impractical in austere environments without a cold chain.

Layering option 2 (mAb + cAd3) would be preferred as the protection windows of each MCM alone align to cover the full deployment phase.

7 Discussions and Potential Next Steps

MCM provide a defensive solution against biological threat agents; however, this is limited if a single MCM does not meet all efficacy requirements in a threat environment. The initial LIMIT concept presents the foundation of a science-informed methodology to define layering rules for vaccines and antibody MCM. The primary goal of these rules is to extend the window of protection afforded by either vaccines or antibody MCM type alone, while avoiding negative interference with how the individual MCM mediate protection.

Empirical data on layering specific vaccine and antibody MCM combinations that is described in MCM labeling or formally recommended by the Advisory Committee on Immunization Practices is always preferred to the use of layering rules. However, in most circumstances this information is not available and the decision to use MCM in combinations would rely on the best judgement of a licensed medical practitioner. In the absence of empirical data and when MCM layering is desirable the layering rules presented in this report for vaccine and antibody MCM provide some guidance based on intuitive immunology principals that are focused on avoiding interference with any processes critical to how MCM protection is mediated. The fundamental process is that if a vaccine and antibody MCM share an antigen there is potential for antibody MCM to interfere with how a vaccine generates immunity. Different vaccine types express or present antigen differently and if vaccine antigen is accessible (i.e., can be bound) to antibody MCM this increases the chances of negative interference. Negative interference between vaccines and antibody MCM could reduce protection against a target pathogen but could also potentially cause serious immune-mediated damage to the host.

A literature review was conducted to identify any instances where vaccine and antibody MCM targeting the same antigen were coadministered; this included instances of pre-exposure and post-exposure prophylaxis in humans or animal models. Only papers detailing with vaccines expressing antigen in a way accessible to antibody MCM were identified and no major trends emerged. Literature should be identified, or studies conducted to validate that vaccines and antibody MCM with a shared antigen can be layered when the antigen is not accessible; for example, in viral vector vaccines that only express antigen in the form of genomic material or nucleic acid vaccines. It is important to note that even in instances were studies determined vaccine and antibody MCM layering did not impact immune response or protection in the short-term, the duration and quality of long-term immune memory can be reduced.[38] Reduction in protective vaccine immune responses from initial layering with antibody MCM can potentially be overcome by providing a booster immunization at a later time.[39]

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[38] EH Boxall *et al*, "Long-term persistence of immunity to hepatitis B after vaccination during infancy in a country where endemicity is low." *Journal of Infectious Disease* 190 (2004).
[39] A Zanetti *et al*, "Does immunoglobulin interfere with the immunogenicity to Pasteur Mérieux inactivated hepatitis A vaccine?" *Journal of Hepatology* 26 (1997).

The LIMIT concept could be used to inform rational design of both MCM and MCM requirements. Rational MCM design conducted with layering rules in mind might focus on selection of different antigen or use of specific vaccine platforms that express target antigen in a way that is not accessible to antibody MCM. Innovative solutions could also include administration of viral vectored or nucleic acid constructs that concomitantly encode for vaccine antigen and monoclonal antibody. This is an emerging strategy but has recently been published for chikungunya virus using a nucleic acid platform and presented at a conference for Pseudomonas aeruginosa using an adenovirus vector platform.[40] [41] MCM layering could also impact requirements setting for MCM development programs. Considered from the perspective of this initial LIMIT concept, layering of MCM can expand protection windows. This could be considered in setting requirements for the time to protection and the duration of protection.

This initial LIMIT concept considers MCM layering during predeployment to extend the window of protection against biological threat agents during deployment. Potential next steps could include MCM administration during deployment in response to exposure to a biological threat agent. Layering of MCM could potentially improve the efficacy offered by a single MCM alone; for example, post-exposure prophylaxis against maternally acquired hepatitis B using IVIG with a hepatitis B vaccine was higher (94 percent) than either IVIG (71 percent) or vaccine (74 percent) alone.[42] MCM layering in this context could also inform requirements setting for efficacy. Requirements setting for Chemical and Biological Defense Program MCM development programs are not clinically integrated even if the mostly likely scenario is that an individual may receive multiple different MCM for a specific pathogen; for example, post-exposure prophylaxis for anthrax includes administration of a vaccine and antibiotics. Ignoring the potential additive effectiveness benefit of MCM layering can result in setting requirements so stringently that it increases development risk, even when it is not scientifically or clinically necessary.

Future iterations of the LIMIT concept should consider additional MCM types, like small molecule drugs or immunomodulators. Once expanded MCM types are included and rules defined, a prototype LIMIT tool could potentially be developed. A LIMIT tool could be used by medical planners to understand MCM options available and support MCM layering decisions. User inputs may include a specific biological threat agent and mission timelines with the output using MCM layering rules and protection parameters to recommend MCM administration schedules to prepare for biological agent exposure by MCM administration preexposure or planning for forward deployment of appropriate MCM.

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[40] K Muthumani *et al*, "Rapid and Long-Term Immunity Elicited by DNA-Encoded Antibody Prophylaxis and DNA Vaccination Against Chikungunya Virus." *Journal of Infectious Disease* 214 (2016).
[41] S Bougarn *et al*, "" Conference presentation, *Recent Advances and Applications in Adenovirus Vectors*, 23 (2015).
[42] S Schillie *et al*, "" *MMWR Recommendations and Reports* 12 (2018).

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Appendix A Abbreviations and Acronyms

Ad	Adenovirus
APC	Antigen-Presenting Cells
AVA	Anthrax Vaccine Adsorbed
CBRN	Chemical, Biological, Radiological, And Nuclear
CBW	Warfighter Integration Division
DTRA	Defense Threat Reduction Agency
DoD	Department of Defense
EBOV	*Zaire Ebolavirus*
EF	Edema factor
GP	Glycoprotein
HPIV	Human Parainfluenza Virus
HSS	Health Service Support
IVIG	Intravenous Immunoglobulin
LF	Lethal factor
LIMIT	Layered and Integrated Medical Intervention Technologies
mAb	Monoclonal Antibody
MCM	Medical Countermeasures
MVA	Modified Vaccinia Ankara
PA	Protective Antigen
PAMP	Pathogen-Associated Molecular Pattern
pfu	Plaque-forming unit
PRR	Pattern Recognition Receptor
S&T	Science and Technology
TTP	Tactics, Techniques, And Procedures
VSV	Vesicular Stomatitis Virus