Biological Risk Assessment Guidance: Production of mRNA Vaccines

Dr Sarita Groenewald

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1. Background

- Depending on the pathogen (a bacteria or virus) that is being targeted, different vaccine technologies could be used to generate an effective vaccine. There are multiple ways to develop a vaccine[1]. Synthetic IVT mRNA has emerged as a next-generation gene-based vaccine technology with the development of in vitro transcription (IVT) and plasmid DNA methods.
- Researchers at UCT develop and evaluate the efficacy and immunogenicity of messenger RNA (mRNA) vaccines in pre-clinical animal models and clinical trials. In some cases, these mRNA vaccines are developed in collaboration with national and international partners which necessitates the transport or importation of the candidate vaccines for R&D purposes. Trained researchers in registered facilities perform the laboratory procedures.
- mRNA vaccines consist of synthetic in vitro transcribed (IVT) mRNA molecules, encapsulated in lipids to protect the fragile RNA and formulate it as a vaccine. The mRNA vaccine production process is done exclusively in vitro, NO living pathogens are used or produced.
- The mRNA is non-infectious and non-integrating, it can only encode for a single protein/antigen in vivo and is NOT self-replicating, eliminating the risk to human and animal health and the environment. In Figure 1 and Table 1 below, the steps involved in mRNA vaccine production, the biological risk assessment of each step and links to information videos and articles are presented. The Risk Analysis Framework is described in Annexure 1.
- Please refer to the following video for an overview of How mRNA vaccines work (start at 1:35), the World Health Organisation Information document on How vaccines are developed and a review of the principles, delivery and clinical translation of mRNA vaccines for infectious diseases[2].

[1] Understanding Six Types of Vaccine Technologies | Pfizer
[2] Chaudhary, N., Weissman, D. & Whitehead, K.A. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. Nat Rev Drug Discov 20, 817–838 (2021). https://doi.org/10.1038/s41573-021-00283-5

2. Definitions

Antigen	A foreign molecule or substance that triggers an immune response in the body, specifically activating lymphocytes to produce antibodies against it.
mRNA Vaccine	A type of gene-based vaccine that uses synthetic messenger RNA (mRNA) to deliver the instructions/sequences for making a harmless piece of protein identical to one found in a particular pathogen (virus or bacterium) to the vaccinated person or animal's cells. The protein or protein fragment (antigen) will generate an immune response.
Plasmid	Small, circular, double-stranded DNA molecule that naturally exists in bacterial cells and can replicate independently. Researchers can insert a gene of interest into a plasmid, which then replicates the gene when it copies itself. This allows the production of large quantities of the desired gene sequence.
Transcription	The process by which the genetic information in a strand of DNA is copied into a new molecule of messenger RNA (mRNA) that carries the information to make a protein (the target antigen).

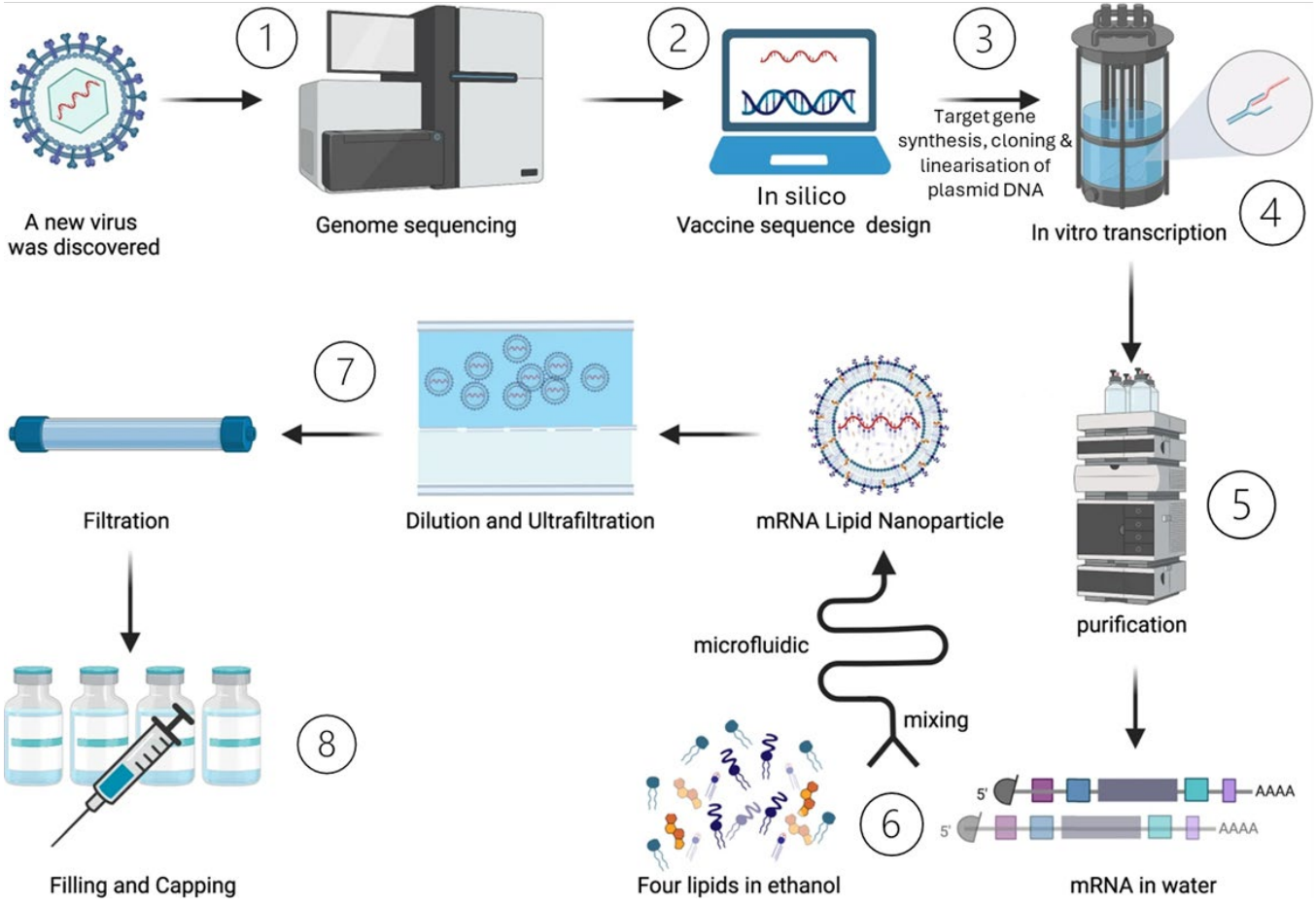

Figure 1. Designing and producing an mRNA vaccine.[3]

[3] Adapted from Fang, E., Liu, X., Li, M. et al. Advances in COVID-19 mRNA vaccine development. Sig Transduct Target Ther 7, 94 (2022). https://doi.org/10.1038/s41392-022-00950-y

Table 1. Production of an mRNA Vaccine: the steps involved and biological risk assessment

Step in mRNA vaccine production (Fig 1)	**Considerations for Biological Risk Assessments**	**Notes and additional information**
1. Isolate and sequence the pathogen's genome	Initial isolation of pathogen under appropriate biosafety level. Early deactivation and subsequent routine rDNA **Biological risk:** Variable (BSL1-3), depending on the pathogen and is limited to the initial stages of the process.	Often not part of independent mRNA vaccine development projects. *Researchers must specify in research approval applications where Step 1 will be/was performed*. Once the pathogen's genome has been sequenced, the sequence data are accessible on public databases or other repositories.
2. In silico analysis and mRNA vaccine design to identify an appropriate antigen and corresponding target gene sequence	In silico sequence analysis and vaccine design (on a computer or via computer software). No organisms are involved. **Biological risk:** None	Antigen selection: the goal is to select one that will trigger an immune response that protects against the pathogen
3. The target antigen's gene sequence is chemically synthesised and cloned using standard recombinant DNA (rDNA) technology. The plasmid DNA containing only the target antigen gene sequences is prepared using *E. coli* lab strains and is subsequently purified. The plasmid DNA is linearised in vitro using restriction enzymes.	Only the target gene(s) is chemically synthesised or amplified from cloned sequences using PCR. The *E. coli* used for cloning experiments are general, routinely used, non-pathogenic laboratory strains. The DNA is purified before the subsequent steps. The described methods are low-risk, routine molecular biology procedures. **Biological risk:** Very low (BSL1)	mRNA Synthesis for the Development of Vaccines and Therapeutics (Merck) Plasmid DNA preparation for mRNA synthesis This and all subsequent steps are conducted with only the gene or gene fragment of the original pathogen. It does NOT self-replicate or behave like the pathogen from which it originates.
4. mRNA production by in vitro transcription: the linear DNA template is transcribed into an mRNA molecule by the enzyme, DNA-dependent RNA Polymerase. The mRNA consists of the coding region, 5' and 3' untranslated regions (UTRs) that regulate mRNA translation, a 5' Cap and a 3' Poly(A) tail.	This enzymatic reaction takes place in a test tube or bioreactor (large scale) and no live organisms are involved. Due to the composition of the reaction medium, the only possible reaction is transcription. **Biological risk:** Very low (BSL1)	mRNA synthesis by in vitro transcription - YouTube
5. Purification of mRNA and removal of DNA template and double-stranded RNA.	Enzymatic, chemical and chromatographic processes. No living organisms are involved, just mRNA molecules. **Biological risk:** Very low (BSL1)	The only biological component after this step is the mRNA that encodes the target antigen's protein sequence.

6. Encapsulation of fragile mRNA in ionisable lipid nanoparticles (LNPs) that deliver the mRNA to the correct location inside the body during vaccination.	LNPs are stable, biodegradable, non-viral delivery vectors that protect the fragile mRNA from degradation and deliver it to the target cells in the body. Good laboratory practices (GLP) are adequate to mitigate potential risks of exposure to LNPs in the laboratory. **Biological risk:** Very low (BSL1)	Lipid nanoparticles
7. LNPs are filtered to remove the non-aqueous solvent and to ensure sterility.		
8. The mRNA-LNP vaccine formulation is used for vaccination. An immune response is triggered in the vaccinated person or animal when the mRNA is translated in vivo.	The mRNA-LNP vaccine formulation is not harmful to human or animal health. **Biological risk:** Low risk of side effects, toxicity, or opportune infections – to be defined and managed clinically.	How are vaccines developed?
Overall biosafety risk conclusion: The production and handling of mRNA-LNP vaccines pose a very low risk of exposure to infectious agents for humans, animals, or the environment.		

Annexure 1. Risk Analysis Framework[4]

- Hazard - is any potential source of harm
- Harm - is an adverse outcome or impact
- Exposure - to a hazard is required before harm can occur

  $\Rightarrow hazard \xrightarrow{exposure} harm$
- Risk - is the probability of harm, of a certain magnitude, occurring

  $\Rightarrow$ Risk = [likelihood of exposure/release x consequence of exposure/release]

		Likelihood of exposure/release				
		Rare	Unlikely	Possible	Likely	Almost certain
Consequences of exposure/ release	Severe	Medium	Medium	High	Very high	Very high
	Major	Medium	Medium	High	High	Very high
	Moderate	Low	Low	Medium	High	High
	Minor	Very low	Low	Low	Medium	Medium
	Negligible	Very low	Very low	Low	Medium	Medium

Risk estimate descriptions and required actions

- **Very low.** If an incident occurred, harm would be very unlikely. Undertake the laboratory activity with the existing risk control measures in place.
- **Low.** If an incident occurred, there would be a small likelihood of harm. Use risk control measures if needed.
- **Medium.** If an incident occurred, harm would result that would require basic medical treatment and/or simple environmental measures. Additional risk control measures are advisable.
- **High.** If an incident occurred, harm would result that would require medical treatment and/or substantial environmental measures. Additional risk control measures must be implemented before the laboratory activity is undertaken.
- **Very high.** If an incident occurred, a permanent, impairing harm or death and/or extensive environmental effects would be likely. Consider alternatives to doing the laboratory activity. Comprehensive risk measures must be implemented to ensure safety.

Likelihood estimates descriptions

- **Rare:** almost impossible to occur (<5%)
- **Unlikely:** not very possible to occur (5-29%)
- **Possible:** might occur (30-69%)
- **Likely:** very possible to occur (70-94%)
- **Almost certain:** highly probable to occur (≥95%)

Consequence estimates descriptions

- **Negligible:** Trivial incident or near miss requiring reporting and follow-up
- **Minor:** Incident with self-limiting consequences
- **Moderate:** Incident that requires medical treatment and/or has insignificant environmental consequences
- **Major:** Incident with potential lost time due to infection but non-permanent consequence and/or limited environmental impact
- **Severe:** Potential fatality or serious illness with permanent disability and/or serious environmental impact

[4] WHO, Risk assessment (Laboratory biosafety manual, 4th edition and associated monographs, 2020) ISBN 978-92-4-001145-8 (electronic version https://www.who.int/publications/i/item/9789240011311)