International Journal of Advanced Trends in Engineering and Technology (IJATET)
Impact Factor: 5.965, ISSN (Online): 2456 - 4664
(www.dvpublication.com) Volume 8, Issue 2, 2023

FORMULATION COMPOSITION AND INFLUENZA VACCINE THERMOSTABILITY BEYOND REFRIGERATED SUPPLY CHAINS

Kannan Vishwanath* & Lloyd Zulu**
* Garwood Innovation Fellow, Garwood Center for Corporate Innovation, Haas School of Business, Berkeley
** World Christian University, Lusaka, Zambia

Cite This Article: Kannan Vishwanath & Lloyd Zulu, "Formulation Composition and Influenza Vaccine Thermostability Beyond Refrigerated Supply Chains", International Journal of Advanced Trends in Engineering and Technology, Volume 8, Issue 2, July - December, Page Number 92-103, 2023.

Abstract:

This analysis evaluates whether influenza vaccine formulation rankings remain consistent across experimental conditions relevant to reducing dependence on refrigerated distribution. Archived experimental data comprise 296 technical measurements aggregated into 101 observed conditions across 14 formulations, covering storage from 2 to 12 weeks at temperatures between 37°C and 48°C. Descriptive summaries and matched comparisons align temperature and duration within screening rounds. Trehalose exceeds corresponding sucrose profiles by averages of 1.51 and 3.98 percentage points in two comparisons, but individual conditions favour both sugars. Sugar and glycine combinations record average advantages of 4.82 and 7.28 percentage points under phosphate buffer in the second round, contrasting with a disadvantage of 10.65 percentage points for the sucrose combination in the first round. Restricting comparisons to complete technical triplicates preserves this directional contrast. These findings refine ingredient-based stabilization expectations by demonstrating conditional performance among complete formulations. The analysis supports formulation selection based on matched exposure conditions and consistent recovery benchmarks, providing a transferable evaluation approach for vaccine development programmes.

Key Words: Influenza Vaccine; Thermostability; Formulation Composition; Antigen Retention; Matched Descriptive Comparisons.

1. Introduction:

Reducing dependence on refrigerated vaccine storage requires formulations that preserve antigen activity under defined thermal exposure. The practical challenge is selecting a composition whose protective performance persists across preparation and storage conditions. Kristensen et al., 2011, explain that stabilization technologies require vaccine specific customization and produce variable outcomes. For influenza vaccines, this makes formulation selection consequential for stockpiling and distribution planning: evidence that an ingredient can protect proteins does not establish that every formulation containing it will retain comparable activity.

Drying offers one approach to preserving biologic activity, but it introduces a balance between protection and processing stress. Removing water can restrict degradation pathways, while freezing and dehydration can damage antigen structure. Preston and Randolph, 2021, identify formulation composition and drying conditions as joint determinants of dried vaccine stability. The relevant analytical question therefore concerns how complete formulations perform under specified conditions, rather than whether an individual excipient is generally considered protective. This distinction matters when laboratory evidence informs the selection of candidates for further development.

Sucrose and trehalose provide related but potentially different forms of protein protection. Vitrification explains stabilization through an amorphous matrix that restricts molecular movement, while water replacement describes protective interactions between sugars and proteins during dehydration. Mensink et al., 2017, show that these explanations depend on molecular interactions, mobility and the stress encountered. Their synthesis does not imply that increasing sugar concentration always improves preservation. Starciuc et al., 2020, further demonstrate competing performance in a model protein system: sucrose better preserved secondary structure during drying, whereas trehalose restricted molecular mobility in the dried formulation. These findings motivate comparisons that distinguish sugar identity, accompanying ingredients and observation conditions.

Glycine adds a different formulation function because its role as a bulking component can complement the protective environment provided by a sugar. Its contribution nevertheless depends on the surrounding composition and preparation process. Thakral et al., 2021, describe dried protein formulations as multicomponent systems in which interactions among excipients and proteins influence stability. Evidence from influenza microneedle development also shows that stabilizer combinations can preserve activity, while their performance depends on manufacturing and storage conditions, as reported by Mistilis et al., 2015. These findings justify examining sugar and glycine profiles as complete compositions. They do not establish an independent glycine effect when adding glycine also changes sugar concentration.

The closest empirical foundation is the influenza subunit vaccine investigation by Flood et al., 2016. The authors screened formulations containing sugars, bulking agents and alternative buffers, developed stable lead formulations and documented extended antigen preservation. Their findings already establish the feasibility

of engineering thermostable influenza vaccine formulations. The present analysis uses their archived measurements for a narrower secondary comparison: whether formulation rankings remain consistent when observations are aligned by temperature and storage duration within screening rounds. Its contribution is a structured comparison of conditional performance within existing experimental evidence, rather than an independent demonstration of vaccine thermostabilization.

Outcome definition is central to this comparison. Retained antigen activity is calculated as mean measured hemagglutinin concentration after storage divided by the corresponding mean before lyophilization, multiplied by 100. This benchmark captures combined processing and storage retention. It allows formulation profiles to be compared on a common relative scale while preserving their individual baselines. Temperature, storage duration and screening round define the comparison conditions. They are used to align observations rather than entered into an estimated moderation model.

This study aims to characterize how retained antigen activity differs among observed sucrose profiles, corresponding sucrose and trehalose formulations, and formulations combining sugars with glycine. The overarching research question is whether formulation rankings remain consistent across the recorded experimental conditions. Specifically, the analysis compares sucrose containing profiles at common observation times, contrasts corresponding sucrose and trehalose compositions, and evaluates sugar and glycine combinations against formulations containing the same sugar alone. These objectives retain the actual composition differences rather than treating each comparison as an isolated ingredient effect.

The empirical setting comprises archived laboratory measurements for H1N1 California/07/2009 X179A subunit vaccine from a United States research programme. The retained sample contains 296 technical measurements aggregated into 101 formulation, temperature and time conditions across 14 formulations. Round 1 observations extend from 2 to 12 weeks at 37°C and 45°C; Round 2 observations cover weeks 2, 4 and 8 at 37°C and 48°C. Descriptive summaries and matched comparisons preserve screening round and use jointly observed storage conditions. This design makes differences in formulation rankings visible without treating technical repeats as independent formulation experiments.

The findings show why this comparative approach matters. Trehalose records higher average retention in two corresponding formulation comparisons, but individual conditions favour both sugars. Sugar and glycine combinations record higher average retention in Round 2, while the Round 1 sucrose combination records lower retention than sucrose alone. These patterns qualify any interpretation based on a universal ingredient ranking. The practical contribution is an evaluation approach that compares complete candidates under common exposure conditions. Its relevance beyond the laboratory setting lies in this comparison principle, which can inform formulation screening while keeping conclusions tied to the antigen platform, preparation conditions and measured outcome.

2. Theory, Conceptual Framework and Research Questions Development:

This analysis approaches influenza vaccine thermostability as a relationship between formulation composition, processing conditions and thermal exposure. Antigen preservation depends on how these elements operate together. Removing water can suppress degradation pathways, but freezing and drying also expose proteins to stresses that protective excipients must counter. Formulation performance therefore reflects both protection during processing and preservation during storage. Preston and Randolph, 2021, explain that dried vaccine stability depends on the balance between these sources of protection and damage. This reasoning directs the analysis toward comparisons among recorded formulations under specified storage conditions.

The conceptual foundation combines vitrification and water replacement theories. Vitrification explains protection through the formation of an amorphous matrix that restricts molecular movement. Water replacement explains how interactions between sugars and proteins can help preserve protein structure as water is removed. These mechanisms are complementary, but their contribution depends on formulation composition and the stress encountered. Mensink et al., 2017, further distinguish local molecular mobility from movement within the wider matrix. Applied to sucrose, this framework suggests that protection depends on the environment created around the antigen rather than concentration alone. We therefore describe retained activity across the observed sucrose formulations without imposing a uniform concentration response.

RQ1: How does retained antigen activity differ among the observed sucrose containing formulations at matched temperatures and storage times within each screening round?

Trehalose provides a related but distinct route to protein stabilization. Although sucrose and trehalose are both disaccharides, their interactions with proteins and residual water can produce different matrix properties. Starciuc et al., 2020, found that trehalose, together with residual water, restricted molecular mobility in dried protein formulations. This finding provides a theoretical basis for differences between the two sugars, but it does not establish a universal ranking for influenza vaccine formulations. This analysis therefore compares sucrose and trehalose profiles where the recorded designs provide corresponding sugar concentrations, accompanying excipients, buffer conditions and storage observations.

RQ2: How does retained antigen activity differ between corresponding sucrose and trehalose formulations under matched conditions within each screening round?

Glycine introduces a structural role that differs from the protective role of the sugars. As a bulking component, it can contribute to the physical organization of a dried formulation, while the accompanying sugar provides an environment that protects the antigen. These functions make formulation performance dependent on the combination of components. Thakral et al., 2021, emphasize that stabilizers interact with other formulation constituents during freezing and drying, so their contribution must be interpreted within the complete formulation. For this analysis, the relevant comparison concerns the observed sugar and glycine combinations rather than an isolated glycine concentration effect.

RQ3: How do formulations combining a sugar with glycine compare with formulations containing the same sugar alone at matched temperatures and storage times within each screening round?

The empirical framework connects these formulation profiles to retained hemagglutinin antigen activity. Flood et al., 2016, provide the formulation compositions and antigen measurements used in the consolidated dataset. Their screening results show that formulation performance differed across storage conditions and preparation rounds. This evidence supports treating temperature, duration and screening round as comparison conditions. Sugar concentrations and the presence of glycine, mannitol, dextran and buffer components define the observed formulation profiles. We compare these profiles within rounds and use common observation times to describe differences in retained activity.

The framework uses directly observed composition measures and a calculated activity outcome. Retained antigen activity equals the mean measured hemagglutinin concentration after storage divided by the mean concentration before lyophilization, multiplied by 100. This measure captures combined processing and storage retention. Each analysis row represents one formulation at one temperature and storage duration, with technical measurements averaged for that condition. Descriptive comparisons examine activity levels, changes over observed storage times and differences between corresponding formulations. The conceptual relationship represents an association between formulation profiles and measured antigen retention, interpreted within the recorded experimental conditions.

3. Data:

This analysis traces formulation compositions and antigen measurements to their published sources and constructs a reproducible sample of observed storage conditions.It defines the measured variables and supports descriptive comparisons among formulation profiles within screening rounds.

3.1 Data Sources, Population, and Sample Construction:

The empirical source is the experimental influenza vaccine dataset accompanying Flood et al., 2016. PATH researchers generated the measurements using H1N1 California/07/2009 X179A subunit vaccine supplied by Novartis Pharmaceuticals. The authors deposited the original workbook, Influenza manuscript data 19AUG16.xlsx, in the Open Science Framework archive registered on 29 August 2016. This analysis extracts elevated temperature measurements from the worksheets labelled Fig 2 and Fig3 and obtains formulation compositions from Table 2 of the accompanying article. The article carries a Creative Commons Attribution licence; the retrieved repository metadata do not identify a separate dataset licence.

The target population comprises influenza subunit vaccine formulations prepared through lyophilization and evaluated under elevated temperature storage. The accessible sampling frame contains seven formulation designs from the first screening round and nine from the second. These laboratory observations originate from a United States research programme and contain no country level sampling. Selection follows the formulation identifiers documented in the published composition table. Liquid screening experiments, refrigerated observations, long duration lead formulation experiments and the additional Round 1 series without a corresponding Table 2 formulation identifier fall outside this extraction.

The analytical unit is a formulation observed at a specified temperature and storage duration. Round 1 contributes observations scheduled at 2, 3, 4, 6, 8 and 12 weeks under 37°C and 45°C storage. Round 2 contributes observations at 2, 4 and 8 weeks under 37°C and 48°C storage. The resulting structure is an unbalanced repeated observation dataset with irregular measurement intervals. Antigen concentrations are measurements at the recorded sampling point, rather than period averages or cumulative flows. The calendar dates of laboratory measurements are not supplied; temporal alignment therefore uses elapsed storage weeks.

Table 1: Sample construction

Stage	Formulation Designs	Conditions	Technical Measurement Slots or Values	Rule
Selected composition frame	16	138	414 slots	Documented formulations crossed with the selected temperatures and observation times
Remove wholly unobserved follow up series	2 removed	24 removed	72 unreported slots	Round 1 formulations F3 and F4 have no reported measurements at the selected follow up times

Stage	Formulation Designs	Conditions	Technical Measurement Slots or Values	Rule
Remove other wholly unobserved conditions	14 remain	13 removed	39 unreported slots	No numeric measurement available for the formulation, temperature and week
Remove explicitly unavailable technical measurements	14 remain	101 remain	7 unavailable values removed	Source cells marked NA
Retained measurement sample	14	101	296 numeric values	Every retained condition contains at least one observed measurement
Final descriptive sample	14	101	101 calculated means	Average available technical measurements within each condition

The final sample comprises 47 conditions from Round 1 and 54 from Round 2. Round 1 contributes 28 conditions at 37°C and 19 at 45°C. Round 2 contributes 27 conditions at each temperature. Of the 101 retained conditions, 96 contain three technical measurements, three contain two and two contain one. The 296 measurements therefore represent technical observations within 101 conditions, rather than 296 independent formulation experiments.

3.2 Data Integration, Cleaning, and Quality Assurance:

Composition records and antigen measurements are linked through formulation identifiers containing both screening round and formulation number. This key prevents formulations sharing the same number across rounds from being merged. Each composition record links to multiple storage observations. The measurement key adds temperature, storage week and technical repeat number. Verification identified no duplicate measurement keys, duplicate condition keys or unmatched formulation identifiers.

The published composition table takes priority when defining excipient content. Source reconciliation corrected two composition discrepancies: Round 1 F3 contains 3% mannitol and no glycine, whereas Round 1 F4 contains 3% glycine and no mannitol. Neither correction changes the retained descriptive sample because these formulations have no observations at the selected follow up times. Other formulation components remain attached to their original identifiers. This treatment preserves the multicomponent character of the formulations, consistent with the component interactions discussed by Thakral et al., 2021.

All 296 retained antigen values were checked against their referenced cells in the original workbook. The audit also recalculated the 101 condition means and their corresponding retention percentages. Source worksheet names and cell addresses preserve the link between each extracted value and its origin. The calculations use numeric measurements rather than copying source summary cells containing unavailable values or spreadsheet errors.

The selected observation grid contains 118 unavailable measurement slots. Of these, 111 belong to 37 conditions without any reported numeric follow up measurement, while seven are explicitly marked NA within otherwise observed conditions. All unavailable slots occur in Round 1. They comprise seven slots for F1, one for F2, 36 each for F3 and F4, 23 for F5, seven for F6 and eight for F7. Round 2 contains all 162 measurements expected from its selected grid.

Unavailable measurements remain distinct from observed zeros. The retained dataset contains 15 numeric zero measurements, which contribute to the relevant condition means. No unavailable antigen value is replaced by zero, interpolated or inferred from adjacent observations. An empty excipient entry in the published composition table is coded zero only when it denotes the absence of that ingredient.

All formulation specific baseline means are positive, so every retained condition has a defined retention denominator. Values above 100% remain unchanged because the outcome expresses measured antigen recovery relative to a measured baseline. No trimming, winsorization or threshold-based outlier deletion is applied. Differences in measurement availability remain visible through the technical measurement count attached to each condition.

3.3 Variable Measurement, Construction, and Validation:

The focal composition measures are sucrose, trehalose and glycine concentrations, expressed as grams per 100 mL of formulation before drying. These variables describe observed formulation inputs. Their interpretation follows the distinction between sugar mediated protein protection and the structural contribution of accompanying excipients. Mensink et al., 2017, explain that sugar protection depends on protein interactions and molecular mobility under the relevant drying and storage conditions. Accordingly, concentrations retain their original units and are interpreted within complete formulation profiles.

The observed antigen measure is hemagglutinin concentration determined through single radial immunodiffusion. Technical measurements are averaged within each formulation, temperature and storage time according to Equation 1.

$$\bar{H}_{ftw} = \frac{\sum_{r=1}^{n_{ftw}} H_{ftwr}}{n_{ftw}}$$

Here, $\bar{H}_{ftw}$ is mean measured hemagglutinin concentration for formulation f, temperature t and storage week w. The term H_{ftwr} denotes technical measurement r and $n_{ftw}$is the number of available numeric measurements for that condition. Retained antigen activity is calculated using Equation 2.

$$R_{ftw} = 100 \times \frac{\bar{H}_{ftw}}{\bar{H}_{fB}}$$

Here, R_{ftw} is retained antigen activity expressed as a percentage, and $\bar{H}_{fB}$is the mean of the three measurements obtained before lyophilization for formulation f. This denominator makes the outcome a measure of combined processing and storage retention.

Table 2: Variable dictionary

Variable or Indicator	Analytical Role	Definition and Construction	Source and Identifier	Unit and Frequency	Transformation and Direction	Missing Value Rule	Coverage and Limitation
Retained antigen activity	Outcome	Condition mean divided by formulation baseline mean, multiplied by 100	Archived Fig 2 and Fig3 measurements	Percentage at each recorded storage time	Equation 2; higher values indicate greater measured retention	Require an observed condition mean and positive baseline	101 conditions; includes processing and storage retention
Mean measured hemagglutinin	Outcome component	Arithmetic mean of available technical measurements	Original measurement cells	Micrograms per mL at each sampling point	Equation 1; retain numeric zeros	Average available numeric values	101 means from 296 measurements
Baseline hemagglutinin	Scaling benchmark	Mean of three measurements before lyophilization	Baseline cells for each formulation	Micrograms per mL; one baseline per formulation	Positive denominator shared across follow up observations	Require numeric baseline values	Complete for all 16 source formulations
Sucrose concentration	Focal composition measure	Recorded sucrose content	Published Table 2	Grams per 100 mL; fixed within formulation	Original scale; higher values denote more sucrose	Zero denotes ingredient absence	Complete for all 101 retained conditions
Trehalose concentration	Focal composition measure	Recorded trehalose content	Published Table 2	Grams per 100 mL; fixed within formulation	Original scale; higher values denote more trehalose	Zero denotes ingredient absence	Complete for all 101 retained conditions
Glycine concentration	Focal composition measure	Recorded glycine content	Published Table 2	Grams per 100 mL; fixed within formulation	Original scale; higher values denote more glycine	Zero denotes ingredient absence	Complete for all 101 retained conditions
Temperature	Comparison condition	Assigned storage temperature	Original worksheet labels	Degrees Celsius; fixed within storage series	Levels of 37, 45 and 48	Require identified temperature	Highest temperature differs between rounds
Storage duration	Comparison condition	Elapsed weeks at measurement	Original worksheet time labels	Weeks; irregular intervals	Original elapsed time	Require identified week	Observed times span 2 to 12 weeks
Screening round	Experimental grouping	First or second formulation screening exercise	Worksheet and formulation identifiers	Categorical	Compare within round	Require identified round	Round combines several experimental changes
Accompanying composition	Matching information	Mannitol, dextran, phosphate and histidine content	Published Table 2	Grams per 100 mL or millimoles per litre	Preserve original ingredient levels	Zero denotes ingredient absence	Used to identify corresponding profiles
Technical measurement count	Coverage indicator	Number of numeric measurements contributing to a mean	Original measurement cells	Count per condition	Values of one, two or three	Count only observed numeric values	Describes measurement availability

Table 3: Descriptive statistics across retained conditions

Variable	Valid Conditions	Mean	Standard Deviation	Median	Minimum	Maximum
Retained antigen activity, %	101	84.77	22.76	89.72	0.00	113.65
Mean measured hemagglutinin, micrograms per mL	101	58.60	21.86	65.68	0.00	89.37
Baseline hemagglutinin, micrograms per mL	101	67.86	15.70	74.96	45.93	89.59
Sucrose, grams per 100 mL	101	1.32	1.57	1.00	0.00	4.00
Trehalose, grams per 100 mL	101	1.43	1.73	0.00	0.00	4.00
Glycine, grams per 100 mL	101	0.83	1.35	0.00	0.00	3.00
Temperature, degrees Celsius	101	41.45	4.99	37.00	37.00	48.00
Storage duration, weeks	101	4.84	2.78	4.00	2.00	12.00

These summaries weight each observed condition equally. Formulations with more recorded follow up conditions consequently appear more often in the pooled statistics. Baseline and composition values repeat across the corresponding conditions.

Sugar identity remains separate from sugar concentration. Starciuc et al., 2020, document differences between sucrose and trehalose in dried protein systems, supporting their treatment as distinct formulation characteristics. Measurement validation here consists of source reconciliation, unit checks, denominator checks and recalculation of means and ratios.

3.4 Analytical Framework, Identification, and Reproducibility:

The analysis is descriptive. It examines observed retention profiles within screening rounds and compares formulations at common temperatures and storage times. Means, sample standard deviations, medians and ranges summarize the measured distributions. Technical measurements contribute to condition means and are not treated as independent formulation experiments.

Sucrose formulation comparisons use the available observations for each profile. Corresponding sucrose and trehalose comparisons use three Round 2 pairs: formulations F1 and F4, F2 and F5, and F3 and F6. These pairs preserve corresponding sugar concentrations and accompanying excipients. Each pair contributes six matched temperature and time conditions, producing 18 paired comparisons.

Comparisons involving glycine use the observed sugar and glycine profiles against sugar alone within the same round and buffer system. The matched contrasts comprise Round 1 F6 against F1 and Round 2 F2 against F1, F5 against F4, and F8 against F7. These comparisons contain 28 matched condition pairs. They compare complete formulations because the sugar concentration also differs between the combined and sugar alone profiles.

Differences in retained activity are expressed in percentage points. Each matched contrast subtracts one formulation percentage from the corresponding comparator percentage at the same temperature and week. Only jointly observed conditions enter a contrast. No later measurement substitutes for an earlier observation, and no value is carried forward across storage times.

Screening rounds remain separate because round membership coincides with changes in vaccine lot, drying conditions, target antigen concentration and other formulation inputs. Storage duration also differs between rounds. The framework therefore uses the experimental variation actually recorded rather than treating all observations as interchangeable. This approach is consistent with Preston and Randolph, 2021, who emphasize that drying conditions and formulation characteristics jointly shape vaccine stability.

The audit trail links each measurement to its original worksheet cell, each composition to its published formulation identifier and each calculated outcome to its baseline denominator. Missing measurements remain documented alongside the retained sample. These links align source definitions, experimental timing and transformations across the descriptive comparisons.

4. Methods:

4.1 Research Design and Comparison Strategy:

This analysis uses a descriptive secondary analysis of archived influenza vaccine experiments. The analytical unit is a formulation observed at a specified storage temperature and elapsed week. The outcome is retained hemagglutinin antigen activity relative to its formulation specific concentration before lyophilization. The sample construction and measurement definitions appear in Table 1 and Table 2, respectively.

The comparison strategy addresses three questions: how sucrose containing profiles differ, how corresponding sucrose and trehalose formulations compare, and how sugar and glycine combinations perform against formulations containing the same sugar alone. Useful variation comes from observed composition differences within screening rounds and repeated measurements under recorded storage conditions. Comparisons preserve temperature and elapsed storage time wherever corresponding observations exist.

Screening rounds remain separate because round membership coincides with changes in vaccine lot, drying conditions, target antigen concentration and other formulation inputs documented by Flood et al., 2016. Matching therefore establishes comparability on selected recorded conditions rather than identifying an isolated causal ingredient effect. Comparisons involving glycine concern complete formulations because glycine inclusion also coincides with a change in sugar concentration.

Technical measurements are averaged within each condition rather than treated as independent formulation experiments. Repeated storage observations also share formulation characteristics and baseline denominators. This distinction follows the separation between observational units and independent experimental replication explained by Lazic et al., 2018. The analysis consequently interprets differences as descriptive contrasts within the observed experiments.

4.2 Outcome Construction and Matched Contrast Specification:

Technical measurement means are calculated using Equation 1, and retained activity is constructed using Equation 2. The latter uses the mean of three measurements before lyophilization as the denominator. This benchmark captures combined processing recovery and subsequent storage retention. For each formulation, the mean across a specified set of observed storage conditions is calculated using Equation 3.

$$\bar{R}_{fC} = \frac{\sum_{c \in C} R_{fc}}{m_C}$$

Here, $\bar{R}_{fC}$ is mean retained activity for formulation facross comparison set C. The term c identifies a temperature and storage week combination, $R_{fc}$is retained activity for formulation funder condition c, and $m_C$is the number of conditions in the comparison set. For sucrose containing profiles in Round 1, the common set at 37°C comprises weeks 2, 3, 4 and 6. The comparison at 45°C uses week 2, the only observation shared by all four profiles. Round 2 sucrose comparisons use weeks 2, 4 and 8 separately at 37°C and 48°C. These restrictions ensure that profiles within each comparison contribute the same observation times. Pairwise contrasts are calculated using Equation 4.

$$\Delta_{abC} = \frac{\sum_{c \in C}[R_{bc} - R_{ac}]}{m_C}$$

Here, Δ_{abC} is the mean difference in retained activity between comparison formulation b and reference formulation a, expressed in percentage points. The terms $R_{bc}$and $R_{ac}$are their respective retention percentages under the same condition. Only conditions observed for both formulations enter C. Positive differences favour formulation b, while negative differences favour formulation a. Corresponding sugar comparisons use Round 2 pairs F1 and F4, F2 and F5, and F3 and F6. In each pair, sucrose is the reference and trehalose is the comparison formulation. These pairs preserve sugar concentration, accompanying excipients and phosphate buffer. Each contributes six matched conditions covering two temperatures and three storage weeks.

Sugar and glycine comparisons use Round 1 F1 as the reference for F6, and Round 2 F1, F4 and F7 as references for F2, F5 and F8, respectively. The Round 1 contrast contains ten jointly observed conditions: six at 37°C and four at 45°C. Each Round 2 contrast contains six conditions. Differences are also summarized separately by temperature to identify whether pooled averages conceal changes in direction.

4.3 Analytical Implementation and Descriptive Estimation:

The analysis uses 296 numeric technical measurements aggregated into 101 observed conditions across 14 formulations. Computation was performed in Python 3.12.14 using the standard library modules zipfile, xml.etree. Element Tree, json, collections and statistics. The procedures are deterministic.

Processing begins by reading the original workbook cells and linking measurements to formulation identifiers, temperature, storage week and technical repeat number. Composition records are reconciled against the published formulation table. Numeric measurements are then grouped by condition, averaged and divided by the corresponding baseline mean. Unrounded values enter all calculations; reported summaries are rounded to two decimal places.

Missing technical measurements do not contribute to condition means. A condition remains eligible when at least one numeric measurement is available. Entirely unobserved conditions are omitted from descriptive summaries and matched contrasts. No interpolation, imputation or carrying forward of measurements is applied. Numeric zeros remain observed values.

Each retained condition receives equal weight in descriptive summaries. Technical measurements receive equal weight within their condition, but conditions with three measurements do not receive greater weight than conditions with one or two. Matched conditions also receive equal weight in Equation 4. Formulations with more observed conditions therefore contribute more frequently to pooled summaries, whereas corresponding profiles contribute identical condition sets within each matched comparison.

Descriptive estimation comprises valid counts, arithmetic means, sample standard deviations, medians, minima and maxima. Sample standard deviations use the number of observations less one as the denominator. Pairwise summaries report the mean difference, the minimum and maximum observed differences, and the number of conditions favouring the comparison formulation. These ranges describe observed variation rather than sampling uncertainty.

Values above 100% remain unchanged because retention is calculated relative to a measured baseline. All baseline means are positive. No trimming, winsorization or outcome threshold determines eligibility.

4.4 Verification, Sensitivity Analysis and Reproducibility:

Verification checks assess the correspondence between source measurements, calculated means and reported retention percentages. All retained numeric measurements are compared with their referenced source cells. Condition means and baseline normalized outcomes are recalculated. Formulation identifiers are checked for unmatched records, while measurement and condition keys are checked for duplicates. These procedures distinguish repeated technical observations from duplicate records.

Two exploratory sensitivity analyses assess specific features of the observed sample. The first restricts pooled summaries to conditions containing three numeric technical measurements. For pair wise comparisons, both formulations must contain three measurements at the matched condition. This restriction evaluates whether incomplete technical replication changes the direction or magnitude of the descriptive contrasts.

The second sensitivity analysis restricts sugar and glycine comparisons to weeks 2 and 4 while retaining the matched temperatures within each round. This provides a common early observation window and evaluates whether the comparison directions depend on later follow up measurements. The restriction changes the comparison sample rather than the retention definition or weighting procedure.

Reproducibility rests on the archived source workbook, formulation identifiers, source cell references, missing measurement records and the construction rules stated in Equations 1 to 4. The analytical workbook records raw measurements, formulation compositions, calculated outcomes and measurement availability. Together, these materials allow the descriptive summaries and matched comparisons to be reconstructed while preserving their interpretation as comparisons among observed formulation profiles.

5. Findings:

This analysis examines retained antigen activity across sucrose formulations, corresponding sucrose and trehalose profiles, and formulations combining sugars with glycine. Matched comparisons align temperature and storage duration within screening rounds. The results answer the three research questions through observed differences, variation and sensitivity to measurement availability.

5.1 Sample Characteristics and Retained Antigen Activity:

The analytical sample contains 101 formulation, temperature and time conditions derived from 296 technical measurements across 14 formulations. Round 1 contributes observations between 2 and 12 weeks, while Round 2 contributes observations at 2, 4 and 8 weeks. Retained activity expresses mean measured hemagglutinin concentration as a percentage of the corresponding mean before lyophilization. The experimental measurements originate from the archived dataset accompanying Flood et al., 2016.

Across all conditions, mean retained activity is 84.77%, with a standard deviation of 22.76 percentage points and a median of 89.72%. Values range from 0.00% to 113.65%. Five conditions record zero activity, while 18 exceed 100%. Each condition receives equal weight after averaging its available technical measurements.

Table 4: Retained antigen activity by screening round and storage temperature

Screening Round	Temperature °C	Observed Conditions	Mean %	Standard Deviation	Median %	Minimum %	Maximum %
Round 1	37	28	83.17	22.15	88.22	0.00	113.65
Round 1	45	19	63.63	35.60	76.90	0.00	106.76
Round 2	37	27	92.87	8.73	91.96	78.74	110.13
Round 2	48	27	93.22	7.65	93.71	79.03	107.42

Round 1 exhibits greater dispersion, particularly at 45°C, where the mean falls below the median by 13.27 percentage points. Round 2 shows more concentrated distributions, with standard deviations below nine percentage points at both temperatures. The similar Round 2 means describe observed retention over eight weeks and do not establish temperature equivalence.

5.2 Sucrose Formulation Profiles and Retained Activity:

The first research question examines differences among sucrose containing formulations under matched storage conditions. Round 1 comparisons at 37°C use weeks 2, 3, 4 and 6, which are shared by all four sucrose profiles. At 45°C, week 2 provides the common observation. Round 2 profiles share observations at weeks 2, 4 and 8 at both temperatures.

Table 5: Retained activity among sucrose containing formulation profiles

Round	Formulation	Composition	Mean at 37°C %	Mean at Higher Temperature %
Round 1	F1	4% sucrose	90.11	78.03
Round 1	F5	1% sucrose and 3% mannitol	41.12	0.00
Round 1	F6	1% sucrose and 3% glycine	77.54	68.04

Round	Formulation	Composition	Mean at 37°C %	Mean at Higher Temperature %
Round 1	F7	3% sucrose and 1% dextran	89.03	75.34
Round 2	F1	4% sucrose	87.52	88.48
Round 2	F2	1% sucrose and 3% glycine	93.62	92.01
Round 2	F3	3% sucrose and 1% dextran	94.18	95.79

Within Round 1, sucrose with mannitol records the lowest retention. Its mean at 37°C is 48.99 percentage points below sucrose alone and 36.42 percentage points below sucrose with glycine. Sucrose with dextran performs closer to sucrose alone, differing by 1.09 percentage points.

The sucrose and mannitol profile declines from 66.40% at week 2 to zero at week 6 under 37°C storage. Sucrose alone records 94.62% and 86.65% at these observations. The difference therefore reflects both lower retention at the first observed follow up and subsequent loss.

Round 2 produces a different ordering. Sucrose with glycine exceeds sucrose alone by 6.09 percentage points at 37°C and 3.54 percentage points at 48°C. Sucrose with dextran exceeds sucrose alone by 6.66 and 7.31 percentage points, respectively.

RQ1 is answered by differences among complete formulation profiles rather than a uniform sucrose concentration pattern. This interpretation aligns with Mensink et al., 2017, who explain that sugar protection depends on protein interactions, molecular mobility and storage stresses. The observed comparisons do not isolate these mechanisms.

5.3 Corresponding Sucrose and Trehalose Formulations:

The second research question compares corresponding sucrose and trehalose formulations within Round 2. Three pairs preserve sugar concentration, accompanying excipients and phosphate buffer. Each pair contributes six matched conditions covering two temperatures and three storage times.

Table 6: Matched comparisons between trehalose and sucrose profiles

Corresponding Composition	Matched Conditions	Sucrose Mean %	Trehalose Mean %	Mean Difference in Percentage Points	Range if Condition Differences	Conditions Favouring Trehalose
4% sugar alone	6	88.00	89.51	Positive 1.51	Negative 6.61 to positive 8.81	4
1% sugar and 3% glycine	6	92.82	96.79	Positive 3.98	Negative 15.43 to positive 20.12	5
3% sugar and 1% dextran	6	94.99	94.69	Negative 0.29	Negative 12.15 to positive 11.12	3

Trehalose records higher average retention in the sugar alone and glycine containing comparisons. However, every pair includes observations favouring each sugar. The dextran containing profiles have nearly identical overall means despite differences across individual conditions.

Temperature specific summaries qualify the pooled averages. For sugar alone, the mean trehalose difference is negative 1.00 percentage point at 37°C and positive 4.02 percentage points at 48°C. For the glycine combinations, the corresponding differences are negative 1.02 and positive 8.97 percentage points. The average trehalose advantage in these profiles is therefore concentrated at 48°C.

RQ2 identifies a conditional pattern rather than consistent trehalose superiority. Starciuc et al., 2020, distinguish sugar performance during drying from preservation in the dried state. Their evidence provides a relevant interpretation of competing sugar performance, while the present outcome combines processing and storage retention.

5.4 Sugar and Glycine Combinations:

The third research question compares formulations containing 1% sugar and 3% glycine with corresponding formulations containing 4% sugar alone. Comparisons preserve screening round, sugar identity, buffer, temperature and observation time. They concern complete formulation profiles because glycine inclusion coincides with lower sugar concentration.

Table 7: Matched comparisons of sugar and glycine combinations against sugar alone

Round and Buffer	Sugar	Matched Conditions	Sugar Alone Mean %	Sugar and Glycine Mean %	Mean Difference in Percentage Points	Conditions Favouring the Combination
Round 1, phosphate	Sucrose	10	78.44	67.79	Negative 10.65	0
Round 2, phosphate	Sucrose	6	88.00	92.82	Positive 4.82	5

Round and Buffer	Sugar	Matched Conditions	Sugar Alone Mean %	Sugar and Glycine Mean %	Mean Difference in Percentage Points	Conditions Favouring the Combination
Round 2, phosphate	Trehalose	6	89.51	96.79	Positive 7.28	5
Round 2, histidine	Trehalose	6	90.91	91.98	Positive 1.07	4

The Round 1 sucrose and glycine combination records lower retention in nine matched conditions and equal retention in one. Its average difference is negative 10.65 percentage points. The difference remains negative at both temperatures, averaging negative 9.76 percentage points at 37°C and negative 11.98 percentage points at 45°C.

Round 2 produces positive average differences. Under phosphate buffer, sucrose with glycine exceeds sucrose alone by 4.82 percentage points, while trehalose with glycine exceeds trehalose alone by 7.28 percentage points. Each combination records higher retention in five of six matched conditions.

The histidine comparison produces a smaller average difference of positive 1.07 percentage points. This difference averages positive 2.17 percentage points at 37°C and negative 0.02 percentage points at 48°C.

RQ3 therefore identifies variation across experimental settings. Sugar and glycine combinations record higher average retention in Round 2, while the Round 1 sucrose combination records lower retention. This pattern accords with the component interactions discussed by Thakral et al., 2021, and supports interpreting the measured combinations within their experimental settings.

5.5 Sensitivity and Synthesis of the Research Questions:

Restricting the sample to conditions with three technical measurements retains 96 observations. Mean retention changes from 84.77% to 84.80%, a difference of 0.03 percentage points. The pooled mean is therefore insensitive to excluding the five conditions with fewer technical measurements.

For the Round 1 sucrose and glycine comparison, requiring three measurements for both profiles retains nine matched conditions. The average difference changes from negative 10.65 to negative 9.61 percentage points, preserving its direction. All Round 2 matched comparisons already contain three measurements per condition.

Restricting the sugar and glycine comparisons to weeks 2 and 4 also preserves their directions. The Round 1 sucrose difference is negative 13.77 percentage points. Round 2 differences are positive 3.03 percentage points for sucrose under phosphate buffer, positive 8.05 for trehalose under phosphate buffer and positive 4.15 for trehalose under histidine buffer.

The findings establish conditional differences among the observed formulations. Sucrose profiles vary with accompanying ingredients and screening round. Trehalose records higher average retention in two corresponding comparisons, alongside reversals across individual conditions. Sugar and glycine combinations perform better on average in Round 2 but not in Round 1. These results support evaluating complete formulation profiles under defined experimental conditions, consistent with the formulation and process perspective developed by Preston and Randolph, 2021.

6. Discussion:

An ingredient that performs well in one formulation setting can lose its advantage in another. Tables 5 and 7 demonstrate this conditional performance: the sucrose and glycine combination records lower retention than sucrose alone in Round 1 but higher retention in Round 2. The analytical contribution lies in making these changes in formulation ranking explicit through matched temperature and time comparisons. Equation 1 aggregates technical measurements within each condition, while Equation 2 expresses retention against the formulation specific baseline. The resulting comparisons show why pooled ingredient rankings can obscure differences among complete formulations. Kristensen et al., 2011, emphasize that stabilization requires customization and produces variable results across vaccines. This analysis sharpens that argument by showing that even within one antigen platform, formulation rankings depend on the experimental setting.

The measured outcome also changes how the apparent protection should be interpreted. Because Equation 2 uses antigen concentration before lyophilization as its denominator, retained activity combines processing recovery with subsequent storage preservation. The differences in Tables 5 and 6 therefore represent the performance of the complete preparation and storage sequence. They cannot be assigned exclusively to protection during storage. Hansen et al., 2015, distinguish freezing and drying stresses from the challenges of preserving dried vaccines, illustrating why these stages require separate conceptual attention. Although their review concerns live virus vaccines, this distinction helps interpret the present subunit vaccine measurements. The contribution is a clearer correspondence between the retention benchmark and the claim supported by the data: higher measured retention indicates better overall recovery under the recorded conditions, rather than direct evidence of a particular molecular pathway.

The matched sugar comparisons further qualify expectations of trehalose superiority. Table 6 shows higher average trehalose retention in two formulation pairs, but every pair includes observations favouring sucrose. The dextran containing profiles have almost identical overall means despite differences across individual conditions. Ohtake and Wang, 2011, document the protective properties and broad biological applications of trehalose. The present evidence places a practical boundary around translating that protective capacity into an ingredient ranking. Under the common measurement scale established by Equation 2, sugar identity provides an incomplete description of performance unless accompanying excipients, temperature and duration are specified. This interpretation refines the conceptual framework by treating sucrose and trehalose as components of conditional formulation profiles rather than assigning either sugar an invariant protective advantage.

Measurement availability reveals another constraint on interpreting formulation performance. Table 4 summarizes an unbalanced set of observed conditions, whereas Tables 6 and 7 compare jointly observed conditions. Restricting the pooled sample to complete technical triplicates changes mean retention by only 0.03 percentage points, and the Round 1 sucrose and glycine contrast retains its direction. These checks show that incomplete technical replication does not explain the principal observed contrast. They do not replace the information absent from wholly unobserved conditions. Kumru et al., 2014, emphasize the importance of analytical methods that connect vaccine integrity with stability assessment. Here, that principle requires distinguishing measured retention, technical measurement coverage and experimental comparability. The resulting insight is methodological: a stable pooled average and a changing formulation ranking describe different features of the evidence and should inform different formulation decisions.

The international relevance lies in the evaluation approach rather than a demonstrated comparison between countries. Mistilis et al., 2015, found that combinations of stabilizers preserved influenza vaccine activity in a microneedle platform, while their performance depended on formulation and manufacturing conditions. Table 7 adds a contrasting pattern: the sugar and glycine combinations examined here do not outperform sugar alone across both screening rounds. Together, these findings support platform specific selection rather than transferring an excipient combination solely because it succeeds elsewhere. Formulation developers should compare candidates at common observation times, preserve the baseline definition in Equation 2 and examine whether an average advantage persists across the intended temperatures. Tables 5 to 7 provide that decision structure. The theoretical extension is correspondingly bounded: formulation composition remains central, but its meaning depends on the preparation and storage conditions under which retention is measured.

7. Conclusion and Implications:

Reducing reliance on refrigerated vaccine systems requires evidence that formulation performance persists under the conditions it must withstand. This analysis shows that protective ingredients acquire practical meaning through their surrounding composition, preparation setting and thermal exposure. Formulation rankings change across experimental settings, making an average advantage insufficient for candidate selection. The contribution is a matched comparison framework that exposes conditional performance within archived experimental evidence. It refines stabilization theory by showing why ingredient-based expectations require qualification at the level of complete formulations. For development managers, the implication is to prioritize candidates using comparable exposure periods and consistent recovery benchmarks rather than pooled rankings. Screening resources should support comparisons that distinguish performance across preparation settings before advancing a candidate. For policy and procurement, laboratory retention evidence should inform technically specified evaluation requirements rather than broad assumptions about suitability for unrestricted distribution. Operationally, preserving source traceability, documenting unavailable measurements and separating technical replication from independent experiments improve the interpretability of screening decisions. These practices offer relevance across vaccine development programmes because they concern how evidence supports selection, rather than where an experiment occurs. Their potential social value lies in strengthening the evidence used to develop dependable vaccine products and plan their subsequent evaluation.

The archived observations provide opportunities for validation through independent batches, additional antigen platforms and longer observation periods. Balanced experiments that vary composition while holding preparation conditions constant could distinguish ingredient contributions from differences between screening rounds. Measurements immediately after drying, followed by repeated storage assessments, would separate processing recovery from subsequent degradation. Direct structural and physicochemical measurements could test the mechanisms proposed to explain conditional performance. Replication across laboratories and manufacturing scales would assess transferability, while transport simulations and field evaluations could establish whether laboratory retention patterns translate into dependable distribution performance.

References:

1. Flood, A., Estrada, M., McAdams, D., Ji, Y., & Chen, D. 2016. Development of a freeze dried, heat stable influenza subunit vaccine formulation. PLOS ONE, 11, issue 11, Article e0164692. https://doi. org/10.1371/journal.pone.0164692

2. Hansen, L. J. J., Daoussi, R., Vervaet, C., Remon, J. P., & De Beer, T. R. M. 2015. Freeze drying of live virus vaccines: A review. Vaccine, 33, issue 42, 5507-5519. https://doi.org/10.1016/j.vaccine.2015.08.085
3. Kristensen, D., Chen, D., & Cummings, R. 2011. Vaccine stabilization: Research, commercialization, and potential impact. Vaccine, 29, issue 41, 7122-7124. https://doi.org/10.1016/j.vaccine.2011.05.070
4. Kumru, O. S., Joshi, S. B., Smith, D. E., Middaugh, C. R., Prusik, T., &Volkin, D. B. 2014. Vaccine instability in the cold chain: Mechanisms, analysis and formulation strategies. Biologicals, 42, issue 5, 237-259. https://doi.org/10.1016/j.biologicals.2014.05.007
5. Lazic, S. E., Clarke Williams, C. J., & Munafò, M. R. 2018. What exactly is N in cell culture and animal experiments? PLOS Biology, 16, issue 4, Article e2005282. https://doi.org/10.1371/journal.pbio.2005282
6. Mensink, M. A., Frijlink, H. W., van der Voort Maarschalk, K., & Hinrichs, W. L. J. 2017. How sugars protect proteins in the solid state and during drying: Mechanisms of stabilization in relation to stress conditions. European Journal of Pharmaceutics and Biopharmaceutics, 114, 288-295. https://doi.org/10.1016/j.ejpb.2017.01.024
7. Mistilis, M. J., Bommarius, A. S., & Prausnitz, M. R. 2015. Development of a thermostable microneedle patch for influenza vaccination. Journal of Pharmaceutical Sciences, 104, issue 2, 740-749. https://doi.org/10.1002/jps.24283
8. Ohtake, S., & Wang, Y. J. 2011. Trehalose: Current use and future applications. Journal of Pharmaceutical Sciences, 100, issue 6, 2020-2053. https://doi.org/10.1002/jps.22458
9. Preston, K. B., & Randolph, T. W. 2021. Stability of lyophilized and spray dried vaccine formulations. Advanced Drug Delivery Reviews, 171, 50-61. https://doi.org/10.1016/j.addr.2021.01.016
10. Starciuc, T., Malfait, B., Danede, F., Paccou, L., Guinet, Y., Correia, N. T., &Hedoux, A. 2020. Trehalose or sucrose: Which of the two should be used for stabilizing proteins in the solid state? A dilemma investigated by in situ micro Raman and dielectric relaxation spectroscopies during and after freeze drying. Journal of Pharmaceutical Sciences, 109, issue 1, 496-504. https://doi.org/10.1016/j.xphs.2019.10.055
11. Thakral, S., Sonje, J., Munjal, B., & Suryanarayanan, R. 2021. Stabilizers and their interaction with formulation components in frozen and freeze dried protein formulations. Advanced Drug Delivery Reviews, 173, 1-19. https://doi.org/10.1016/j.addr.2021.03.003