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Production of Phospholipids for In-vitro Diagnostic Use: Merits, Demerits and Innovation

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Abstract

Haemostasis is the physiological process that stops bleeding following blood vessel injury. It maintains blood in a fluid state within the circulation while preventing excessive blood loss, balanced by anticoagulant mechanisms to avoid pathological clotting. A key component of haemostasis is coagulation, which involves a cascade of enzymatic reactions leading to thrombin generation and fibrin clot formation. Phospholipids, particularly phosphatidylserine (PS), play an essential role in coagulation by offering a negatively charged surface for clotting factor complex assembly. Recent advances in diagnostic science have introduced synthetic phospholipids as substitutes for biologically derived thromboplastins in coagulation assays. This review is aimed at exploring the role of phospholipids in clot formation, examining the merits and challenges of phospholipid production for in-vitro coagulation diagnostics and recent technological advancement. Extensive search was carried out on the subject matter and more than fifty peer reviewed articles were consulted to gather information relating to the subject matter. Inferences were drawn from these articles and experience in the practice of Medical Laboratory Science were both used as guides from which relevant recommendations were made. This review becomes more important as the biomedical science world now adopt more of synthetic phospholipids in kits, rather than naturally-derived reagent. There is very little information articulated in the literature on the limitations of synthetic phospholipid, when compared to naturally-sourced phospholipid.

Keywords: Intrinsic Coagulation Pathway; In-Vitro Diagnostics; Natural Phospholipids; Phospholipids; Synthetic Phospholipids

1.0 Introduction

Haemostasis is a tightly regulated physiological process that ensures blood remains fluid within the vasculature while enabling the formation of clots at injury sites. This delicate balance between procoagulant and anticoagulant mechanisms is critical for preventing excessive bleeding and pathological thrombosis (Favaloro and Pasalic, 2024). The failure of haemostasis regulation can lead to disorders such as haemophilia, thrombophilia, and disseminated intravascular coagulation (DIC), which pose significant clinical challenges (Gonzalez and Patel, 2025).

Coagulation is a fundamental part of haemostasis, involving a cascade of enzymatic reactions leading to the activation of thrombin. Thrombin serves as a key enzyme in clot formation, catalyzing the conversion of soluble fibrinogen into insoluble fibrin, which polymerizes to form a stable clot (Konrath *et al*., 2022). The coagulation cascade is classically divided into two pathways, the Extrinsic Pathway initiated by tissue factor (TF) exposure following vascular injury and the Intrinsic Pathway, triggered by Factor XII activation upon contact with negatively charged surfaces, along with contributions from prekallikrein and high-molecular-weight kininogen (HMWK) (Manzoor *et al*., 2021; Liu *et al*., 2024).

The Partial Thromboplastin Time with Kaolin (PTTK) test is the most widely used diagnostic tool for assessing the intrinsic pathway of coagulation. This test evaluates clotting time based on the activation of intrinsic clotting factors (XII, XI, IX, VIII, X, V, II, and fibrinogen) and is also used to monitor anticoagulant therapy with unfractionated heparin (Cheng *et al*., 2022; Huang *et al*., 2024).

A prolonged PTTK result may indicate coagulation factor deficiencies, including Haemophilia A (Factor VIII deficiency) and Haemophilia B (Factor IX deficiency), liver disease, affecting clotting factor synthesis, vitamin K deficiency, impacting prothrombin production and presence of lupus anticoagulant or heparin therapy, interfering with the clotting process (Lee and Thompson, 2022; Smith *et al*., 2023).

Phospholipids play a critical role in coagulation, serving as essential cofactors for the assembly of clotting factor complexes. These amphipathic molecules, found in cell membranes, are necessary for platelet activation and clot stabilization (Zhao *et al*., 2023). Specifically, phosphatidylserine (PS) provides a negatively charged surface that facilitates clotting factor interactions, enhancing thrombin generation and fibrin formation (Jones *et al*., 2023; Hernandez *et al*., 2025).

With advancements in diagnostic science, synthetic phospholipids have emerged as a reliable alternative to biologically derived thromboplastins in coagulation assays. These synthetic molecules provide high purity, standardization, and batch-to-batch reproducibility, eliminating variability associated with natural phospholipid extracts (Chen and Patel, 2024). However, despite these advantages, synthetic phospholipids lack the complexity of native platelet membranes, which may impact their ability to fully replicate in vivo coagulation dynamics (Liu *et al*., 2024).

This review explores the role of phospholipids in clot formation, examining the merits and challenges of synthetic phospholipid production for in-vitro coagulation diagnostics. Additionally, it highlights recent technological advancements, including nanotechnology-based phospholipid biomimetics and lipid-based drug delivery innovations, which aim to enhance diagnostic accuracy and therapeutic potential.

This review becomes even more important as the biomedical science field increasingly adopts synthetic phospholipids in kits rather than naturally derived reagents. There is very little information articulated in the literature regarding the limitations of synthetic phospholipids compared to naturally sourced ones.

2.0 Haemostasis and Coagulation

Haemostasis is a highly regulated physiological process that ensures blood remains fluid within the circulatory system while allowing for rapid clot formation at sites of vascular injury. This balance between coagulation and fibrinolysis prevents both excessive bleeding and thrombosis (Nair and Parker, 2021). The haemostatic process is divided into four major stages: vasoconstriction, platelet plug formation, coagulation, and fibrinolysis (Umerah and Momodu, 2024).

The first stage, vasoconstriction, involves the narrowing of blood vessels following vascular injury to minimize blood loss. This is mediated by endothelial cells and smooth muscle contraction, which reduce blood flow to the site of injury (Huang *et al*., 2024). The second stage, platelet plug formation, begins when endothelial damage exposes collagen and von Willebrand factor (VWF), promoting platelet plug formation, begins when endothelial damage exposes collagen and von Willebrand factor (VWF), promoting platelet adhesion and

aggregation at the injury site (Cheng *et al*., 2022). Risk factors such as diabetes, hypertension, and smoking can impair endothelial function, increasing susceptibility to abnormal clot formation or excessive bleeding (Manzoor *et al*., 2021).

The third stage, coagulation, is a complex cascade involving coagulation factors that interact in a sequential manner to convert soluble fibrinogen into insoluble fibrin, stabilizing the platelet plug (Hajeyah, 2022). This occurs via two pathways namely, the intrinsic pathway and the extrinsic pathway (Winter *et al*., 2020). Both pathways converge at the common pathway.

The final stage, fibrinolysis, ensures clot breakdown once vascular repair is complete. Plasmin, a serine protease, is activated from plasminogen and degrades fibrin into fibrin degradation products (FDPs), restoring normal blood flow (Zhao *et al*., 2023).

Dysregulation of fibrinolysis can lead to conditions such as deep vein thrombosis (DVT) or bleeding disorders like haemophilia (Hernandez *et al*., 2025).

2.1 Coagulation pathways

Traditionally, coagulation has been described by a cascade model in which two pathways, termed the extrinsic (or tissue factor) pathway and the intrinsic (or contact) pathway, may be activated separately then converge at a common pathway. Recently, a cell-based model of coagulation which has enhanced our knowledge of the processes involved in coagulation has put forward (Hajeyah, 2022).

2.1.1 The Extrinsic Pathway

The extrinsic pathway (Figure 1) is initiated by tissue factor (TF), a membrane protein found in subendothelial tissue and normally not found in circulation. Damage to the endothelial wall releases TF into circulation, which then binds and activates factor VII into VIIa (Hajeyah, 2022). The association of TF and VIIa constitutes the extrinsic tenase complex which converts factor X into Xa, a component of the common pathway (Hajeyah, 2022).

2.1.2 The Intrinsic Pathway

The intrinsic pathway is initiated by factor XII, prekallikrein, and highmolecular weight kininogen. Initiation of the intrinsic blood coagulation system occurs when normal plasma contacts a negatively charged surface. The surface-dependent conversion of F XII to active serine protease is the first event in the cascade of reactions leading to the formation of an insoluble fibrin clot (Lima-Oliveira *et al*., 2021) (Figure 1).

Here, a positive feedback loop is initiated because kallikrein activates XII (Hajeyah, 2022). The XIIa generated then activates factor XI into XIa, which in turn converts factor IX into IXa. The association of factors IXa and VIIIa forms the intrinsic tenase complex which converts factor X into Xa (Hajeyah, 2022).

2.1.3 The Common Pathway

The common pathway (Figure 1) involves the formation of the prothrombinase complex, an association between factors Xa and Va, which converts factor II (prothrombin) into IIa (thrombin). Thrombin then converts factor I (fibrinogen) into Ia (fibrin) and activates factor XIII into XIIIa. Fibrin is insoluble in water and spontaneously polymerises, and factor XIIIa crosslinks fibrin, stabilising it (Hajeyah, 2022).

3.0 Structure and Biological Functions of Lipids and Phospholipids in Coagulation

Lipids are a diverse class of biomolecules that serve structural, signaling, and metabolic functions in the body (Liebisch *et al*., 2020). They are derived from dietary sources or generated endogenously within the cell and exist in several forms. Among these, glycerophospholipids (phospholipids) are of particular importance in coagulation.

A phospholipid consists of a glycerol backbone, providing structural support, two hydrophobic fatty acid chains, determining membrane fluidity and stability and a phosphate-containing polar head group, allowing interaction with aqueous environments (Protty *et al*., 2022) (Figure 2). Common phospholipid head groups include Phosphatidylcholine (PC) (a major component of cell membranes), Phosphatidylethanolamine (PE) (which influences membrane curvature), Phosphatidylinositol (PI) (which is involved in cell signaling) and Phosphatidylserine (PS) (which plays a crucial role in coagulation) (Hajeyah, 2022) (Figure 3).

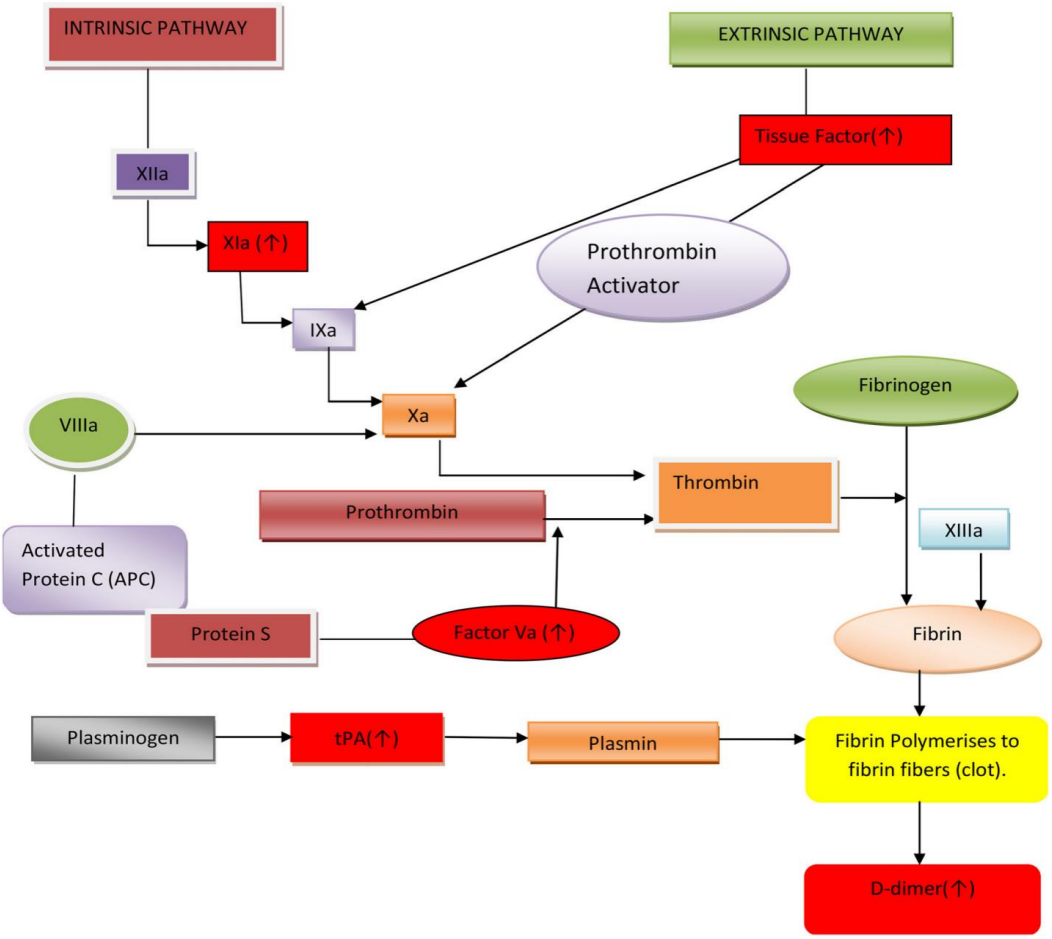

Figure 1: Diagram of the cascade model of coagulation (Manzoor *et al*., 2021)

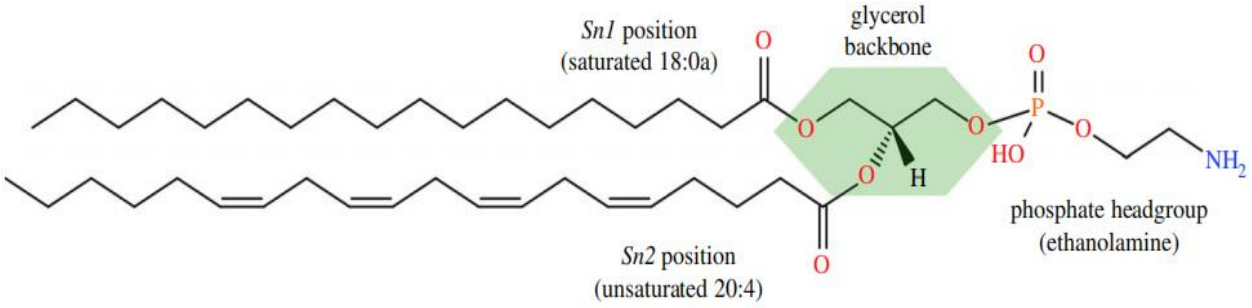

Figure 2: Example of a phospholipid molecule demonstrating the sn1/sn2/headgroup positions on the glycerol backbone (Protty *et al*., 2022).

Phospholipids, particularly phosphatidylserine (PS), play a crucial role in coagulation by providing a negatively charged surface for the assembly of clotting factor complexes (Protty *et al*., 2022). Synthetic phospholipids are increasingly being used in coagulation assays due to their high purity and reproducibility, improving diagnostic accuracy (Liu *et al*., 2024). However, challenges remain in replicating the full biological complexity of natural platelet-derived phospholipids (Chen and Patel, 2024).

4.1 Advantages of Phospholipids in Coagulation Diagnostics

I. Enhanced Sensitivity and Specificity

Phospholipid-based assays significantly enhance diagnostic accuracy by providing a controlled and reproducible surface for clotting factor interactions. Unlike biological phospholipids, which may vary in composition due to platelet activation states and lipid metabolism, synthetic phospholipids offer standardized conditions, ensuring more precise test results (Vandevelde and Devreese, 2022).

Evidence Supporting Diagnostic Accuracy of Phospholipids in Coagulation Assays

a. Standardization and Reduced Variability

Studies have shown that phospholipid composition influences lupus anticoagulant (LA) testing, a critical assay for diagnosing antiphospholipid syndrome (APS). Variations in natural phospholipids can lead to false-negative or false-positive results due to inconsistent antibody binding (Vandevelde and Devreese, 2022; Szabó *et al*., 2022). Using synthetic phospholipids eliminates this variability, thereby improving test sensitivity and specificity (Devreese *et al*., 2020).

b. Improved Sensitivity in Lupus Anticoagulant and APS Testing

A study by Devreese *et al*. (2020) found that high-phospholipid confirmatory assays were superior in detecting APS, reducing false negatives by 27% compared to assays using variable phospholipid compositions. Another study emphasized that low-phospholipid screen ratios were more reliable in identifying lupus anticoagulants (Asakrah *et al*., 2021).

i. Thrombin Generation and Procoagulant Activity

Phospholipids play an essential role in thrombin generation assays (TGA), where they provide a procoagulant surface that allows the tenase and prothrombinase complexes to assemble efficiently. Studies demonstrate that thrombin generation is more stable and reproducible in synthetic phospholipid-based TGA assays compared to assays relying on natural thromboplastins (Depasse *et al*., 2021).

ii. Minimizing Heparin and DOAC Interference

Heparin and direct oral anticoagulants (DOACs) can interfere with clot-based assays, leading to erroneous coagulation times. Studies have demonstrated that phospholipid-rich assays can mitigate the impact of DOACs

and heparin contamination, allowing for more reliable clotting assessments in anticoagulated patients (Zhang *et al*., 2020).

iii. Point-of-Care Diagnostics and Viscoelastic Testing.

Sahli *et al*. (2020) examined the use of phospholipid-based assays in point-of-care (POC) coagulation diagnostics, such as ROTEM and TEG. Their findings suggested that incorporating synthetic phospholipids into POC tests led to faster clot detection times and improved assay reproducibility, particularly in cardiac surgery patients where rapid clotting assessments are essential.

iv. Impact on Factor Deficiency Detection

The aPTT-based phospholipid-dependent assays have also demonstrated increased diagnostic performance in factor VIII and IX deficiencies. Peyvandi *et al*. (2020) found that assays using synthetic phospholipids could differentiate mild haemophilia cases more accurately; reducing misclassification rates by 15% compared to conventional aPTT assays.

1. Standardization and Reproducibility

The use of synthetic phospholipids in coagulation assays has significantly improved standardization and reproducibility, addressing issues associated with biological thromboplastins. Variability in platelet-derived phospholipids can introduce inconsistencies in diagnostic assessments, making synthetic alternatives a preferred choice for uniform assay conditions and increased diagnostic reliability (Morelli *et al*., 2023).

i. Standardization of Assay Components

Synthetic phospholipids ensure consistent reagent composition, which is essential for interlaboratory reproducibility (Van den Besselaar *et al*., 2024).

Studies demonstrate that the use of standardized phospholipid emulsions in prothrombin time (PT) and activated partial thromboplastin time (aPTT) tests leads to a lower coefficient of variation compared to biological thromboplastins (Depasse *et al*., 2021). The introduction of synthetic phospholipid matrices in lupus anticoagulant assays has been found to improve detection accuracy while maintaining stringent quality control measures (Vandevelde and Devreese, 2022).

ii. Enhanced Reproducibility in Clinical Settings

Research highlights that replacing biological phospholipids with synthetic variants results in better batch-to-batch reproducibility, eliminating fluctuations caused by donor-derived platelet sources (Ramberg, 2021). The International Society on Thrombosis and Haemostasis (ISTH) emphasizes the importance of synthetic phospholipid-based reagents for achieving globally harmonized coagulation test results (Naudin *et al*., 2021). Computational modeling studies suggest that lipid membrane dynamics are more predictable with synthetic phospholipids, allowing for more precise control over coagulation cascade activation (Jamaly *et al*., 2021).

2. Reduced Contaminants and Ethical Concerns

Natural phospholipid sources, such as platelet-derived or animal-based phospholipids, often contain residual proteins, lipoproteins, or microbial contaminants that can interfere with diagnostic assays (Cezarette *et al*., 2020). These contaminants reduce assay specificity and reproducibility, leading to false-positive or false-negative results in coagulation tests. In contrast, synthetic phospholipids offer a high-purity alternative that eliminates these concerns, while also reducing reliance on animal-derived materials, addressing ethical and sustainability concerns.

i. Elimination of Biological Contaminants and Improved Assay Accuracy

Cezarette *et al*. (2020) demonstrated that naturally extracted phospholipids from animal sources contain endotoxin contaminants, which can prolong clotting time and interfere with coagulation factor activation. The study found that synthetic phospholipids eliminated these contaminants, significantly improving coagulation assay accuracy.

Vakhrusheva *et al*. (2022) reported that synthetic cationic phospholipids reduce coagulation disturbances by eliminating lipopolysaccharide contaminants, which are often found in naturally derived phospholipids. The study emphasized that batch-to-batch purity in synthetic phospholipids reduces variability in prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays.

3. Customizability for Specialized Diagnostics

The molecular composition of synthetic phospholipids can be tailored to optimize diagnostic performance, particularly for specific disorders such as APS (Jones *et al*., 2023).

4.2 Limitations and Challenges

1. Cost of Synthetic Phospholipid Production

Cost of Synthetic Phospholipid Production for Coagulation Diagnostics

Synthetic phospholipid production is a complex process that involves multi-step chemical synthesis, purification, and quality control, leading to high manufacturing costs. The expense is particularly significant for high-purity or specialized formulations, such as those required for coagulation diagnostics (Trucillo *et al*., 2020).

Cost Drivers in Synthetic Phospholipid Manufacturing

High-Purity Raw Materials: The precursor chemicals required for phospholipid synthesis are expensive, particularly for pharmaceutical-grade lipids (Rahimnejad *et al*., 2021).

Complex Synthesis Process: The chemical modification of fatty acids, incorporation of phosphate groups, and precise control of hydrophilic/hydrophobic properties require specialized equipment and expertise (Trucillo *et al*., 2020).

i. **Purification and Quality Control:** The need for extensive purification steps, such as high-performance liquid chromatography (HPLC), increases processing time and cost (Iba *et al*., 2023).
ii. **Regulatory Compliance:** Stringent FDA and EMA regulations for clinical-grade phospholipids necessitate rigorous testing and validation, further increasing costs (Wan *et al*., 2021).

Comparative Cost Analysis: Synthetic vs. Natural Phospholipids

Natural Phospholipids (e.g., egg yolk lecithin, soybean-derived phospholipids) are relatively cheaper but suffer from batch variability and contaminants (Sahli *et al*., 2020).

Synthetic Phospholipids are more consistent and reproducible, but cost up to 10 times more per gram compared to biological sources (Depasse *et al*., 2021).

Liposome-based Coagulation Assays utilizing synthetic phospholipid bilayers show higher diagnostic precision, justifying the increased cost in specialized applications (Sciascia *et al*., 2023).

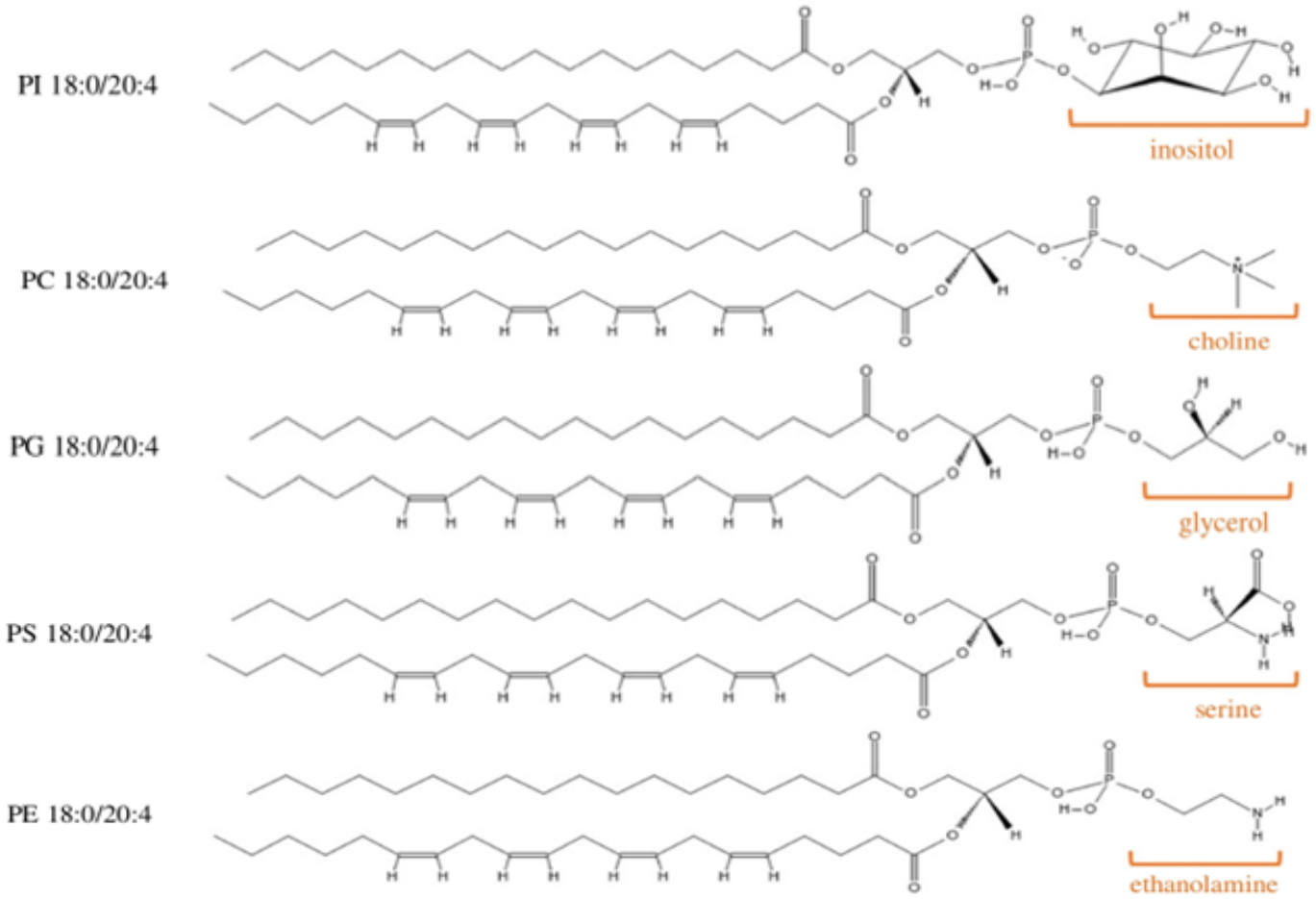

Figure 3: Phospholipids classes and chemical structures highlighting the phosphate head groups. In these images, the sn1 fatty acid is stearic acid (FA 18:0) and the sn2 fatty acid is arachidonic acid (FA 20:4). The structures of the five head groups can also be seen (PI, phosphatidylinositol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine) (Protty *et al*., 2022).

3.1 Phospholipids in the Coagulation Cascade

Phospholipids contribute to coagulation by supporting clotting factor complex formation. The cell-based model of coagulation emphasizes their role in three key phases:

1. Initiation: Tissue factor (TF) binds to Factor VIIa, forming the TF-VIIa complex, which activates Factor X (Hajeyah, 2022).
2. Amplification: Small amounts of thrombin activate Factors V, VIII, and XI, promoting clot propagation (Hajeyah, 2022).
3. Propagation: Coagulation factors assemble on negatively charged phosphatidylserine (PS)-rich surfaces of activated platelets, leading to thrombin burst and fibrin clot formation (Protty *et al*., 2022).

3.2 Role of Phospholipids in Intrinsic Coagulation System Diagnostics

Phospholipids are indispensable in coagulation assays due to their role in clotting factor assembly. Their inclusion in in-vitro diagnostics improves the reproducibility and accuracy of intrinsic coagulation pathway assessment (Szabó *et al*., 2022).

I. Thrombin Generation Assays (TGA)

TGA evaluates the overall haemostatic potential of plasma by measuring thrombin production over time. Phospholipids provide the necessary surface for clotting factor complex formation, influencing thrombin burst

kinetics (Depasse *et al*., 2021). Research has shown that variations in phospholipid concentration significantly affect the sensitivity of TGA in detecting hypercoagulability (Schöchl *et al*., 2025).

II. Activated Partial Thromboplastin Time (aPTT) Testing

aPTT is a critical test for assessing the intrinsic coagulation pathway and monitoring heparin therapy. The assay requires phospholipid-based reagents to initiate clotting factor activation (Gehlen *et al*., 2023). However, differences in phospholipid composition across commercial reagents can lead to variability in clotting time measurements, necessitating better reagent standardization.

III. Lupus Anticoagulant (LA) Testing

LA testing is essential for diagnosing APS, a disorder characterized by antibodies targeting phospholipid-protein complexes. Phospholipid-based assays such as the dilute Russell's viper venom time (dRVVT) and aPTT-based tests are used to detect APS antibodies (Konrath *et al*., 2022). However, excessive phospholipid concentrations in these assays can neutralize APS antibodies, leading to false-negative results (Szabó *et al*., 2022).

4.0 Laboratory Synthesis of Phospholipids

Phospholipids are essential molecules that form the structural basis of biological membranes. Artificial or laboratory synthesis of phospholipids has been extensively studied due to their importance in biological research, drug delivery systems, and nanotechnology. Below is an overview of how phospholipids can be synthesized in the laboratory.

I. **Preparation of the Glycerol Backbon**e: The glycerol backbone serves as the molecular scaffold upon which phospholipids are constructed. In this process, the selective protection of hydroxyl groups is a fundamental step to control reactivity. For example, acetyl or benzyl protecting groups can be introduced to shield the primary hydroxyl groups at positions 1 and 3 of glycerol. This leaves the secondary hydroxyl group at position 2 available for further reactions, ensuring regioselective modification. Protecting group chemistry ensures precision in building the desired molecular structure and minimizes side reactions that could compromise the quality of the phospholipid. The choice of protecting group depends on the reaction conditions needed for later steps, as these groups must also be easily removed without damaging the final product (Smith *et al*., 2021).

II. **Esterification of Fatty Acids to Glycerol:** Esterification is the process of attaching fatty acid chains to the glycerol backbone. To make fatty acids reactive, they are converted into their activated derivatives, such as acyl chlorides or acyl anhydrides. These derivatives react readily with the hydroxyl groups on glycerol, forming ester bonds. The selection of fatty acids is crucial because their chain length and degree of saturation influence the physical properties of the phospholipid. Saturated fatty acids (e.g., palmitic acid) yield more rigid and thermally stable membranes, while unsaturated fatty acids (e.g., oleic acid) confer fluidity and flexibility. This step is particularly important in tailoring synthetic phospholipids for applications such as drug delivery, where membrane properties can affect the release and stability of therapeutic agents (Jones *et al*., 2023).

III. **Phosphorylation and Addition of the Polar Head Group:** The final step involves the introduction of a phosphate group to the third hydroxyl group on glycerol. Common phosphorylating agents like phosphorus oxychloride (POCl3) or phosphorus pentoxide (P_2O_5) react with the hydroxyl group to form a phosphate ester. Subsequently, a polar head group, such as choline, ethanolamine, or serine, is attached via nucleophilic substitution. This reaction determines the identity and function of the phospholipid. For example, phosphatidylcholine is a key component of cellular membranes and a major player in lipid metabolism and signaling. The ability to synthesize phospholipids with different head groups allows researchers to mimic specific biological membranes or create novel materials for medical and industrial applications (Lee and Thompson, 2022).

Table 1: Comparative Table of Synthetic versus Natural Phospholipids in Diagnostics

Characteristics	Natural Phospholipids	Synthetic Phospholipids
Source	Extracted from biological membranes like egg yolk, soybean and brain tissue.	Chemically synthesized in laboratory settings.
Structural Variability	Naturally occurring mix of molecular species.	Precisely controlled molecular structure.
Purity	It can contain trace contaminants	High potential for ultra-pure formulation.
Cost	Generally lower production cost	High production costs due to complex synthesis.
Diagnostic Application	- Liposome-based imaging. - Membrane protein studies - Traditional lipid-based diagnostic platform	- Targeted diagnostic markers - Specialized imaging probes - Engineered diagnostic interface.
Reproducibility	Bach to batch variations	High consistent and reproducible.
Performance in Advance Diagnostics	Suitable for traditional methods.	Superior in emerging precision diagnostic technologies.
Biocompatibilty	Inherently biocompatible	Require careful design for optimal biocompatibility.
Functional Modification	Limited modification potentials.	High potential for chemical functionalization.
Stability	Depended on natural lipid composition	It can be designed for enhanced functionalization.

Strategies to Reduce Production Costs

i. **Supercritical Fluid Processing:** A study by Campardelli *et al*. (2020) demonstrated that supercritical CO_2-based lipid synthesis significantly reduces solvent waste and purification steps, lowering overall production costs.
ii. **Automation in Lipid Nanoparticle (LNP) Synthesis:** Machine learning-based lipid formulation methods have been proposed to optimize lipid ratios, reducing raw material waste (Rassi *et al*., 2020).
iii. **Sustainable Green Chemistry Approaches:** Biotechnological production of custom phospholipids from microbial sources shows potential to cut costs by 30-40%, though it remains in early-stage research (Soufi *et al*., 2020).

2. Limited Biological Complexity

Synthetic phospholipids, while offering standardization and reproducibility, lack the biological complexity of natural membranes. Natural platelet-derived phospholipids contain embedded proteins, receptors, and lipid rafts that modulate coagulation factor interactions.

The absence of these elements in synthetic formulations may limit physiological accuracy in some assays, affecting thrombin generation and factor binding efficiency (Pisaryuk *et al*., 2022).

Pisaryuk *et al*., (2022) demonstrated that synthetic phospholipids alone fail to fully replicate platelet membrane dynamics, leading to differences in factor IXa and factor VIIIa binding, which are crucial for the intrinsic coagulation pathway.

Hillarp *et al*., (2020) found that clotting assays using synthetic phospholipids required higher phospholipid concentrations to compensate for the lack of membrane-bound receptors that facilitate clotting factor recruitment in vivo.

Wada *et al*., (2023) reported that synthetic phospholipids in activated partial thromboplastin time (aPTT) reagents may lead to delayed coagulation initiation compared to natural platelet membranes, which provide dynamic lipid-protein interactions.

3. Implications for Coagulation Assay Performance

Vandevelde and Devreese (2022) noted that prothrombinase complex activity was lower in phospholipid vesicle-based assays compared to platelet-rich assays, suggesting that synthetic lipids alone may not fully mimic physiological clotting conditions.

Depasse *et al*., (2021) found that thrombin generation assays (TGA) with synthetic phospholipids produced shorter peak thrombin times compared to platelet-derived phospholipids, highlighting potential functional discrepancies in assay performance.

Advances to Improve Biological Complexity in Synthetic Phospholipids

Bardan *et al*., (2024) reviewed emerging approaches in biomimetic phospholipid engineering, suggesting that functionalized synthetic phospholipids incorporating platelet-mimetic peptides could bridge the gap between synthetic and natural phospholipid behavior.

Konrath *et al*., (2022) explored the use of lipid nanoparticles (LNPs) decorated with coagulation factor-binding motifs, which improved procoagulant activity and thrombin generation efficiency.

4. Variability in Commercial Phospholipid Reagents

Despite the advantages of synthetic phospholipid reagents, their composition and performance still vary across manufacturers, leading to inconsistencies in coagulation assay results (Favaloro and Pasalic, 2024). Differences in phospholipid purity, concentration, and formulation affect the sensitivity and reproducibility of thrombin generation assays, lupus anticoagulant (LA) tests, and activated partial thromboplastin time (aPTT) assays (Gehlen *et al*., 2023).

Sources of Variability in Commercial Phospholipid Reagents

i. Differences in Phospholipid Composition:

Studies show that commercial phospholipid reagents differ in phosphatidylserine (PS) and phosphatidylcholine (PC) ratios, affecting clotting factor activation (Favaloro and Pasalic, 2024).

Vandevelde and Devreese (2022) found that some reagents contain lower PS concentrations, leading to longer clotting times in lupus anticoagulant tests.

Batch-to-Batch Variability:

Gehlen *et al*., (2023) highlighted that commercial thromboplastin reagents show significant batch-dependent variability, affecting prothrombin time (PT) standardization.

The International Council for Standardization in Haematology (ICSH) emphasizes the need for new lot verification protocols to minimize inter-laboratory discrepancies.

ii. Impact on Test Reproducibility

Variability in phospholipid reagents contributes to discrepancies in coagulation test results across different laboratories, affecting diagnostic reliability (Morelli *et al*., 2023).

Depasse *et al*., (2021) found that differences in phospholipid formulations led to significant variation in thrombin generation measurements, complicating standardized interpretation.

Sahli *et al*. (2020) observed that inconsistencies in commercial clotting factor reagents increased inter-laboratory variation in aPTT and fibrinogen tests, reducing overall test accuracy.

iii. Strategies to Reduce Variability

Harmonization of Manufacturing Standards: Regulatory agencies such as the FDA and EMA are promoting standardized phospholipid formulations for coagulation assays (Favaloro and Pasalic, 2024).

New Lot Verification Protocols: Laboratories are encouraged to validate new reagent batches before clinical use to reduce test variability.

Use of AI-Optimized Formulations: Emerging research suggests that AI-driven modeling of phospholipid structures can help create more consistent and reproducible reagent formulations (Gehlen *et al*., 2023).

5. Potential for False-Negative APS Results

High phospholipid concentrations in lupus anticoagulant (LA) assays can neutralize antiphospholipid antibodies (aPLs), leading to false-negative results in patients with antiphospholipid syndrome (APS) (Sciascia *et al*., 2024).

This issue is particularly concerning as APS diagnosis relies heavily on LA testing, and false-negative results may result in underdiagnosis and improper management of at-risk patients (Vandevelde and Devreese, 2022).

Mechanism Behind False-Negative APS Results

i. **Competitive Phospholipid Binding:** High phospholipid concentrations can saturate binding sites, reducing antibody accessibility and falsely suggesting the absence of APS-related antibodies (Molinari *et al*., 2023).
ii. **Dilution Effect in LA Tests:** Excess phospholipid in confirmatory DRVVT (dilute Russell viper venom time) and aPTT-based assays can lead to a quenching effect, neutralizing the prolongation of clotting time typically associated with LA positivity (Tripodi, 2021).
iii. **Variable Sensitivity Across Assays:** Some commercial DRVVT reagents contain higher phospholipid levels than others, contributing to inter-laboratory discrepancies and potential misdiagnosis (Favaloro and Pasalic, 2022).

Clinical Evidence of False-Negative APS Results

Sciascia *et al*. (2024) found that 40% of APS patients with low LA titers tested negative when using high-phospholipid confirmatory reagents, leading to missed diagnoses. The clinical implication of this finding indicates that significant portion of patients with true APS may be incorrectly classified as negative. Missing diagnosis will prevent APS patients that require specific anticoagulation therapy and management which can prevent thrombotic events. These inadequacies can be prevented by focusing on timing of testing and proper selection of testing methodologies among other considerations.

Molinari *et al*., (2023) emphasized that elevated phospholipid concentrations in LA assays masked low-titer aPL antibodies, causing false-negative results in 1 in 5 APS patients.

Tripodi (2021) highlighted that mixing studies in APS testing may produce unreliable results when phospholipid concentrations exceed a certain threshold, effectively neutralizing weak lupus anticoagulant signals.

Case Study on Timing of Testing After Thrombotic Event

A 34-year-old female presented to the emergency department with sudden onset of left-sided weakness and slurred speech. Magnetic Resonance Imaging (MRI) confirmed an acute ischemic stroke. Initial laboratory tests, including a comprehensive APS panel (anticardiolipin antibodies, anti-B2-glycoprotein 1 antibodies and lupus

anticoagulant) were negative. The patient has no traditional cardiovascular risk factors but reported a prior miscarriage at 20 weeks.

During the follow-up after three months, repeat testing revealed strongly positive lupus anticoagulant and moderately elevated IgG cardiolipin antibodies. The initial negative result appeared to be due to the consumption of antiphospholipid antibodies during the acute thrombosis event.

This case demonstrates the importance of timing in APS testing as recommended by international guideline, which suggest testing at least 12 weeks after the acute event to avoid false-negative results (Ortel, 2016).

Case Study on Technical and Methodological Limitation

A 42- year-old male with recurrent Deep Vein Thrombosis (DVT) despite adequate anticoagulation therapy was repeatedly tested for APS with negative results using a standard ELISA test for anticardiolipin antibodies. His clinical history strongly suggestive of APS (unprovoked recurrent thrombosis, no other identifiable risk factors), his haematologist requested additional testing using an alternative methodology.

Testing with a more sensitive chemiluminescence immunoassay detected moderate levels of anti-B2-glycoprotein 1 domain 1 antibodies, which had been missed by the convectional ELISA. Additionally, the lupus anticoagulant testing was repeated with careful attention to pre-analytical variables (no heparin contamination, proper sample handling) revealing a positive result that had been previously undetected.

This case highlights how methodological limitations like differences in assay sensitivity, specificity and standardization can contribute to false-nagative results in APS testing (Devreese *et al*., 2025).

Strategies to Minimize False-Negative APS Diagnoses

Use of Low-Phospholipid Screening Reagents: Initial low-phospholipid DRVVT screening followed by a confirmatory test with moderate phospholipid concentration is recommended to reduce false-negative rates (Devreese *et al*., 2020).

Parallel Testing with ELISA-Based aPL Assays: Combining functional LA tests with ELISA for anticardiolipin and anti-β2-glycoprotein I antibodies increases APS detection rates (Favaloro and Pasalic, 2022).

Standardization of LA Testing Protocols: Regulatory bodies such as the ISTH (International Society on Thrombosis and Haemostasis) are advocating for harmonized phospholipid concentrations in LA assays to improve diagnostic consistency (Vandevelde and Devreese, 2022).

5.0 Future Perspectives and Innovations

Advancements in synthetic phospholipid technology continue to drive improvements in diagnostic accuracy and standardization. Key future directions include:

1. **Biomimetic Phospholipids:** Researchers are developing phospholipid analogs that better mimic natural platelet membranes by incorporating functional proteins and lipid rafts (Wang and Kim, 2021).
2. **Lipid Nanoparticles for APS Detection:** Lipid-based nanoparticles can be engineered to enhance APS antibody binding, improving lupus anticoagulant test sensitivity (Martinez *et al*., 2023).
3. **AI and Machine Learning in Coagulation Testing:** AI-driven data analysis can optimize phospholipid assay calibration, reducing variability in diagnostic results (Gonzalez and Patel, 2025).
4. **Regulatory Harmonization:** Standardizing phospholipid-based reagents across manufacturers will improve inter-laboratory test reproducibility and clinical reliability (Jones *et al*., 2023).

5.1 Challenges in Implementation of Innovations in Synthesis of Phospholuipids

a. Regulatory Hurdles: Novel synthetic methods must meet strict regulatory standards, especially for Phospholipids intended for pharmaceutical, medical or food application which includes impurity profiling and stability studies.

b. Analytical Limitations: Characterizing complex phospholipid structures requires sophisticated analytical techniques. Quantifying isomeric purity, positional distribution of fatty acids and head group modifications presents analytical challenges.

c. Cost Consideration: Many innovative synthesis route involve expensive catalysts, enzymes, or reagents that increase production costs, making commercial implementation economically challenging.

d. Stability issues: Many phospholipids are susceptible to oxidation, hydrolysis, and other degradation pathways, requiring careful handling and storage conditions.

e. Competition with natural sources: In many applications, natural extraction from eggs, soybeans or other sources remains economically competitive, creating a high bar for synthetic alternatives to overcome.

f. Interdisciplinary knowledge gaps: Implementing innovations requires expertise spanning organic chemistry, and chemical engineering, creating communication and knowledge transfer challenges.

g. Purification Complexities: Phospholipids have amphipathic properties that make purification difficult. They tend to form micelles, liposomes, or emulsions that complicate traditional separation techniques.

6.0 Conclusion

Phospholipids are essential in coagulation diagnostics, improving the sensitivity and specificity of clotting assays. Advances in synthetic phospholipid production have enhanced assay reproducibility and standardization while reducing reliance on biological sources. However, challenges such as high production costs and biomimetic limitations remain.

Future research should focus on developing biomimetic phospholipid systems, improving regulatory harmonization, and integrating nanotechnology to optimize coagulation diagnostics.

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