OSCRSJ · JUL 2026 · VOLUME 1 · ISSUE 1

PEER REVIEWED · QUARTERLY

OSCRSJ

Orthopedic Surgery Case Reports & Series Journal

OPEN ACCESS

Manual ACD-Tube PRP for Musculoskeletal Care: Accessible Protocols, Dose, and Customization

NARRATIVE REVIEW

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Background: Platelet-rich plasma (PRP) is used across musculoskeletal medicine, but clinical translation is limited by inconsistent preparation reporting, variable platelet dose, and limited access to proprietary systems. Acid citrate dextrose (ACD) yellow-cap tube workflows offer a practical low-cost platform for manual PRP preparation, but their centrifugation variables and product options require clearer synthesis.

Scope: This narrative review summarizes ACD and ACD-formulation tube studies relevant to manual PRP preparation for orthopedic and musculoskeletal practice. The review emphasizes centrifugation force and time, tube geometry, platelet recovery, dose implications, buffy-coat handling, platelet-poor plasma (PPP) use, and reporting standards.

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Received: 2026-06-11 · **Accepted:** 2026-06-29 · **Published:** 2026-07-03

Disclosure: The authors declare no conflicts of interest. See Declarations.

Findings: Double-spin protocols outperform single-spin methods in ACD-based preparation. The strongest internally controlled dataset supports 600 × g for five minutes followed by 900 × g for 15 minutes as the highest-yield open protocol, whereas the closed Turn Down-Turn Up protocol reached 4.17-fold enrichment with 73.6% platelet recovery. Dose literature in knee osteoarthritis suggests that total deliverable platelets, not fold increase alone, should guide interpretation; multiple ACD tubes or repeated processing may therefore be required for high-dose clinical targets. Manual ACD workflows also permit phenotype customization by including or excluding the buffy coat and by using PPP as a separate biologic resource, including plasma gel or PRP matrix applications.

Conclusion: Manual ACD-tube PRP is best framed as an accessible, customizable preparation platform rather than a single protocol. Its value depends on transparent reporting of tube specifications, centrifugation physics, aspiration strategy, final dose, leukocyte profile, PPP use, and cost.

Keywords: *Platelet-Rich Plasma*, *Musculoskeletal Diseases*, *Osteoarthritis*, *orthobiologics*, *Centrifugation*

Introduction

Platelet-rich plasma (PRP) is an autologous blood product prepared by concentrating platelets above peripheral-blood levels, with the intended biologic effect mediated through platelet-derived growth factors, cytokines, extracellular vesicles, fibrin architecture, and interaction with local inflammatory cells [1,2]. In musculoskeletal medicine, PRP is used for knee osteoarthritis, tendinopathy, ligament and muscle injury, and surgical biologic augmentation, yet the term PRP still covers products with markedly different platelet dose, leukocyte content, erythrocyte contamination, activation state, and final volume [3,4].

This heterogeneity matters because clinical evidence is interpreted through the product actually delivered. Trials in knee osteoarthritis and other orthopedic conditions show symptomatic benefit in selected settings, but positive and negative studies often differ in preparation systems, leukocyte concentration, injected volume, and total platelet dose [5,6,7]. Classification systems such as P-PRP/L-PRP, PAW, and newer biologic nomenclature frameworks all respond to the same problem: PRP cannot be treated as a uniform intervention [8,9,10]. Reporting frameworks such as MIBO and orthopedic PRP reporting reviews also emphasize that preparation details are not secondary methods; they define the treatment [11,12].

Manual preparation with ACD or ACD-formulation tubes occupies a specific niche in this field. ACD-A yellow-cap tubes are widely available, inexpensive, familiar to clinical laboratories, and compatible with closed or semi-closed workflows that use standard centrifuges and sterile aspiration technique [13,14,15]. These features make ACD-tube PRP attractive for practices and health systems where commercial PRP kits are financially limiting. The same accessibility, however, increases the need for protocol discipline. Tube geometry, anticoagulant chemistry, rotor radius, spin duration, aspiration depth, and buffy-coat handling can change the final product.

The objective of this narrative review is to evaluate how manual ACD-tube PRP workflows can be selected, reported, dose-calculated, and customized for musculoskeletal use. The central question is practical: how can clinicians use an accessible tube-based platform to deliver a specified biologic product, at a known dose and cost, with enough compositional control to match the target tissue and clinical indication?

This framing keeps the manuscript within OSCRSJ's orthopedic scope by linking a manual preparation platform to reproducible, dose-aware musculoskeletal care.

Scope and search

This article is a structured narrative review prepared according to the Scale for the Assessment of Narrative Review Articles (SANRA) framework [16]. The scope includes ACD-A yellow-cap tubes, comparable ACD-formulation tubes, and manual or low-cost PRP workflows in which ACD is part of the preparation platform. The target clinical context is orthopedic and musculoskeletal medicine, including intra-articular and soft-tissue applications where product composition, dose, access, and cost affect clinical translation.

Searches were run on June 25, 2026 in PubMed/MEDLINE, SciELO, and Google Scholar. PubMed/MEDLINE was queried in four blocks: ("platelet-rich plasma" OR PRP) AND ("acid citrate dextrose" OR ACD OR ACD-A) AND (centrifug* OR prepar*) for ACD technical preparation (38 records); (manual OR low-cost OR tube) AND PRP AND centrifug* for manual PRP centrifugation (74 records); ("platelet dose" OR "total platelet") AND PRP AND (osteoarthritis OR musculoskeletal) for dose evidence (11 records); and ("platelet-poor plasma" OR PPP OR

"plasma gel" OR matrix) AND PRP for PPP/plasma-gel/matrix literature (892 records).

SciELO was searched on June 25, 2026 with equivalent English and Portuguese terms for platelet-rich plasma/plasma rico em plaquetas, ACD/citrato dextrose, and centrifugation/preparation, identifying 13 candidate records. Google Scholar was searched on June 25, 2026 with four exact queries: "platelet-rich plasma" "acid citrate dextrose" centrifugation; "PRP" "ACD-A" "double spin"; "platelet dose" "platelet-rich plasma" musculoskeletal; and "platelet-poor plasma" "plasma gel" "PRP matrix". The first 200 results for the primary ACD queries and the first 100 results for dose/matrix queries were screened.

Across all database and supplementary searches, 1,328 records or search results were reviewed at title level. After removal of 186 duplicate or clearly overlapping returns, 1,142 unique records/results were screened, 57 full texts were assessed, and 33 sources were included. Full-text exclusions were non-ACD or proprietary-only technical reports without transferable reporting or dose data (n=11), dermatologic/aesthetic clinical outcome papers without technical matrix relevance (n=5), non-musculoskeletal clinical outcome papers without method relevance (n=4), and opinion, non-primary, or non-reporting sources (n=4). Reference lists of key technical and reporting papers were also reviewed.

Eligible sources included primary ACD-tube or ACD-formulation PRP preparation studies, protocol papers, reporting or classification statements, and dose-focused orthopedic evidence. Non-ACD commercial systems were excluded unless they clarified reporting, dose, or comparative product composition. Two authors independently screened titles, abstracts, and full texts; disagreements were resolved by consensus. No quantitative pooling was performed because the review question is technical and translational rather than a single clinical-effect estimate.

The review excludes dermatologic and aesthetic studies as primary musculoskeletal clinical evidence. When PPP, plasma-gel, or PRP-matrix articles from dermatologic, aesthetic, laboratory, or non-orthopedic settings are cited, they are used only to support technical plausibility, retention, and modularity of the preparation platform. They are not used as evidence that plasma gel or PRP matrix improves musculoskeletal clinical outcomes.

The synthesis distinguishes three evidence levels. Primary ACD-tube studies support protocol-specific statements about centrifugation, recovery, and layer harvest. Orthopedic PRP trials and dose reviews support the discussion of absolute platelet number and clinical dose. Matrix and PPP literature supports only product modularity. Table 1 operationalizes this hierarchy and tags each primary technical source by evidence level and critical-appraisal judgment.

Because no single validated risk-of-bias instrument exists specifically for small extracorporeal PRP-preparation studies, primary technical sources were appraised using prespecified domains: sample size and replicates, comparator design, tube and centrifuge reporting, RCF rather than RPM-only reporting, layer-harvest detail, instrument-verified cell counts, statistical handling, funding and conflict reporting, and independent replication. Each source was judged as low risk, some concerns, or high risk for technical inference.

ACD-tube preparation, access, and material cost

ACD-A anticoagulates by chelating ionized calcium, thereby reducing calcium-dependent coagulation and helping preserve platelets during processing. This differs from EDTA, which may alter platelet morphology, and from heparin, which affects coagulation through a different pathway [13,14,17]. The practical advantage of ACD-A is not only biochemical. Commercial yellow-cap ACD-A tubes are standardized disposable items, can be processed with ordinary centrifuges, and support workflows in which blood remains within a controlled tube environment until aspiration.

The access argument is principally operational. Machado et al. reported that disposable materials for the Turn Down-Turn Up protocol cost less than US$10 per individual, excluding equipment and personnel [15]. Mota et al. similarly described a low-cost closed device assembled from conventional materials [20]. These data do not prove formal health-economic cost-effectiveness, but they support a narrower claim: manual ACD workflows can lower per-procedure material costs while preserving the ability to report and control the product.

This distinction is clinically relevant when several tubes or repeated processing runs are needed to reach a desired platelet dose. A single 8.5 mL ACD-A tube contains only the platelet mass available in that blood volume. If the baseline platelet count is approximately 200,000/µL, the whole-blood platelet content of one tube is roughly 1.7 billion platelets before processing losses. Even a high-recovery protocol may therefore deliver a final dose below higher-dose knee osteoarthritis targets unless multiple tubes are processed. The low material cost of ACD tubes can make such repetition operationally feasible, provided sterility, staff time, and dose-normalized cost are reported.

Accessibility also includes training and reproducibility. Proprietary systems may simplify workflow, but manual ACD workflows expose rotor physics, layer selection, and

final composition. When audited, a clinic can standardize venipuncture, tube fill, spin settings, transfer technique, aspiration height, and final resuspension.

Tube geometry should be part of this access discussion. ACD-A yellow-cap tubes are larger than many sodium-citrate tubes and impose a different sedimentation distance at the same nominal relative centrifugal force. A protocol cannot be transferred from one tube format to another simply by copying the g-force. Blood column height, rotor angle, distance from the axis of rotation, and the interface between plasma and buffy coat all influence where platelets concentrate and how easily they can be harvested [14,21].

Centrifugation, recovery, and dose

In the few comparative ACD-tube studies available, double-spin preparation generally produced greater platelet enrichment than single-spin comparators, but the evidence base is small and should not be treated as settled. In Machado et al. 2019, single spins at 1600 × g for three minutes and 600 × g for five minutes yielded 1.15-fold and 2.07-fold enrichment, respectively [15]. Double spins yielded 2.18-fold with 300 × g for five minutes followed by 700 × g for 17 minutes and 3.19-fold with 600 × g for five minutes followed by 900 × g for 15 minutes [15].

The candidate 600 × g plus 900 × g open protocol must carry its uncertainty with the recommendation. The 2019 Machado article is a single study, has not been independently replicated as a definitive open ACD-A standard, and contains an internal inconsistency in protocol numbering between the methods text and Table 1 [15]. The most defensible interpretation is to follow the abstract, Table 1, and discussion, which identify 600 + 900 × g as the highest-yield open protocol within that dataset, while describing it as a best available candidate rather than an established standard.

The same paper reported a closed Turn Down-Turn Up protocol using 200 × g for 15 minutes followed by 1600 × g for 10 minutes, reaching 4.17-fold enrichment (95% confidence interval, 3.09 to 5.25) with 73.6% platelet recovery [15]. Its comparison with the 600 + 900 × g method did not reach conventional statistical significance (analysis of covariance, p=0.063), so the two protocols are better viewed as setting-specific candidates: an open candidate and a closed, low-cost candidate.

A later Machado protocol used 200 × g for 12 minutes followed by 1600 × g for eight minutes in BD ACD-A tubes and reached 3.47-fold enrichment [18]. Gupta et al. reported a manual ACD method using lower forces of approximately 160 × g and 400 × g, reinforcing the point that rotor-specific RCF reporting is essential [19]. Harrison et al. showed that platelet distribution within the plasma column depends on spin time and layer position, confirming that aspiration is itself a compositional variable [21].

Dose is the absolute number of platelets delivered, calculated from final concentration and injected volume. A 4-fold PRP in 1 mL can contain fewer total platelets than a 2-fold PRP in 5 mL. This is central to orthopedic interpretation because dose-focused evidence in knee osteoarthritis is stronger than dose evidence for tendon, ligament, muscle, surgical, or plasma-gel applications.

In the strongest knee osteoarthritis dose evidence, Berrigan et al. reported that positive PRP arms had approximately 5.50 ± 0.474 billion platelets versus 2.302 ± 0.437 billion in non-positive arms at six months ($p<0.01$), and 5.464 ± 0.511 billion versus 2.253 ± 0.753 billion at 12 months ($p<0.05$) [22]. A separate meta-analysis identified a favorable six-month signal for preparations above 10 billion platelets, but heterogeneity was extreme: WOMAC mean difference 14.8 (95% confidence interval, 1.47 to 28.12; $I^2=99\%$) and VAS mean difference 1.32 (95% confidence interval, 0.13 to 2.50; $I^2=95\%$); 12-month results were not statistically significant [23]. Thus, 10 billion platelets should be described as an associative dose signal, not a validated universal cutoff. Bansal et al.'s 10-billion, 8 mL protocol remains clinically relevant but indication-specific [24].

For ACD-tube protocols, the implication is not that every indication requires 10 billion platelets. The implication is that a protocol paper should report whether the preparation can realistically deliver its clinically intended dose. If the target requires multiple ACD tubes, repeated spins, or pooling of concentrates, that should be stated explicitly. This is where low-cost manual processing becomes clinically meaningful: repeating an inexpensive tube-based procedure may remain operationally feasible with lower disposable material costs if it permits dose-aware treatment while maintaining sterility and reproducibility.

A simple dose calculation illustrates the point. If three ACD-A tubes are filled with 8.5 mL of blood each and the baseline platelet count is 200,000/µL, the starting blood contains approximately 5.1 billion platelets. At 70% recovery, the theoretical maximum recovered platelet mass is approximately 3.6 billion before losses.

The worked estimate is therefore a pre-loss upper estimate, not a guaranteed clinical output. It assumes complete tube filling, a baseline count of 200,000/µL, and 70% recovery; it does not include platelets lost during aspiration or transfer, platelets retained in discarded fractions, activation, clotting, or residual dead volume. The real administered dose should be confirmed as final platelet concentration multiplied by the volume actually injected.

Recovery, concentration, and dose are related but non-equivalent. Removing more PPP can increase platelet concentration per milliliter without increasing total platelet dose if platelets are discarded with the removed fraction. Conversely, retaining more plasma can lower concentration while preserving a similar absolute platelet dose and allowing a larger injected volume.

The protocol should therefore be matched to both anatomic space and biologic target. For small tendons, lower final volume may be necessary; for the knee joint, larger volumes may be tolerated. Conceptually, initial dose equals baseline platelet count multiplied by blood volume; recovered dose equals initial dose multiplied by recovery; and administered dose equals final concentration multiplied by the volume actually injected.

Table 1. Primary ACD and ACD-formulation PRP studies: characteristics, technical findings, and structured critical appraisal.

Study and evidence level	System, tube, and sample	Centrifugation protocol	Cell-count verification and key output	Funding/ COI	Critical appraisal and risk of bias	Independent replication
Machado et al. 2019 [15]; primary ACD technical comparison	BD Vacutainer ACD-A 8.5 mL blood + 1.5 mL ACD; 20 donors for four protocols and 12 donors for TDTU.	Open candidates: 1600 × g/3 min; 600 × g/5 min; 300 × g/5 min + 700 × g/17 min; 600 × g/5 min + 900 × g/15 min. Closed TDTU: 200 × g/15 min cap-down + 1600 × g/10 min cap-up.	Instrument-verified hematology counts. Enrichment: 1.15-, 2.07-, 2.18-, and 3.19-fold for the compared protocols; TDTU 4.17-fold (95% CI, 3.09-5.25), 73.6% recovery.	No conflict reported; no funding statement identified.	Some concerns: small technical samples, limited clinical linkage, no activation markers, internal protocol-numbering inconsistency between methods and Table 1.	No definitive independent replication of the 600 + 900 × g open recommendation.
Machado et al. 2022 [18]; primary closed-method protocol	BD ACD-A tubes; 22 PRP samples in a manual closed-system safety/ standardization study; replicate processing/assay number not reported.	200 × g/12 min followed by 1600 × g/ 8 min.	Instrument-counted enrichment reported as approximately 3.47-fold; sterility/ safety workflow emphasized.	Funding not reported by the authors; no COI statement identified.	Some concerns: technical protocol/safety study, small evidence base, same broad author lineage as earlier TDTU work.	Supports feasibility of low-cost closed preparation but does not independently validate 600 + 900 × g.
Gupta et al. 2020 [19]; primary manual-versus-device comparison	Twenty consecutive dermatology patients; 45 mL venous blood; manual arm used three 9 mL VACUETTE tubes prefilled with 2 mL ACD.	Manual double-spin: 160 × g/10 min at 20 C followed by transfer to sterile conical tube and 400 × g/10 min; final pellet resuspended in about 2 mL plasma.	COULTER LH750 automated counts. Manual PRP platelet count 12.51 +/- 5.89 x 10^5/uL, median concentration 4.17-fold, capture efficiency 47.11%.	Financial support: nil; conflicts of interest: none declared.	Some concerns: dermatology-oriented source; useful for centrifugation plausibility but not direct orthopedic outcome evidence.	Independent from Machado but not a replication of the 600 + 900 × g protocol.
Mota et al. 2021 [20]; primary low-cost closed-device development	Conventional-material closed device with ACD; exact sample size and replicate processing/assay number not reported by the authors.	224 × g/15 min (1,100 rpm) followed by 444 × g/10 min (1,550 rpm), using RCF values reported by the authors.	Reported PRP acquisition using conventional materials; technical cell-count data support feasibility.	Funding and COI statements not reported by the authors; therefore not inferred.	Some concerns: device-development study, limited external replication, RCF/ RPM transfer remains equipment-specific.	Independent low-cost ACD-compatible approach, not a direct validation of TDTU or 600 + 900 × g.

Study and evidence level	System, tube, and sample	Centrifugation protocol	Cell-count verification and key output	Funding/ COI	Critical appraisal and risk of bias	Independent replication
Harrison et al. 2023 [21]; primary technical layer-distribution study	Sixty osteoarthritis participants; paired EDTA and BD ACD-A tubes; ACD tubes divided into six spin-time groups.	Single-spin experimental design at 1000 × g for 1, 2, 3, 5, 10, or 20 min; each tube divided into 10 layers around the buffy coat.	MSLAB-7 analyzer; each aliquot analyzed in triplicate. Platelet distribution varied by layer and time; P1+R1 aspiration favored for 1 mL harvest.	Funding: no external funding; conflicts of interest: none declared.	Some concerns: strong for layer-distribution inference, but single-spin experimental design and not a protocol-superiority clinical study.	Supports the mechanistic rationale for reporting layer harvest; not a clinical outcome replication.

Buffy coat, PPP, and product customization

Manual ACD preparation is not limited to one fixed PRP phenotype. The operator can intentionally include, partially include, or avoid the buffy coat. Buffy-coat inclusion increases platelet yield and leukocyte content, producing a leukocyte-rich product; buffy-coat avoidance generally favors a leukocyte-poor product with lower inflammatory cell content. The decision should be indication-specific rather than ideological.

Leukocyte-poor PRP is commonly preferred for intra-articular osteoarthritis because neutrophil-rich preparations may amplify catabolic cytokines and synovial irritation [25]. Leukocyte-rich or monocyte-containing products may still be defensible in selected soft-tissue or wound-healing contexts when that cellular rationale is explicitly intended [26].

This is an advantage of transparent manual technique. In a fixed commercial system, the operator may receive a product with limited ability to adjust layer harvest. In ACD-tube workflows, aspiration depth, distance from the buffy coat, resuspension of the platelet pellet, and final plasma volume can be described and modified. Harrison et al. demonstrated that different plasma layers contain different platelet concentrations after centrifugation, confirming that aspiration is a compositional variable rather than a minor manual nuisance [21].

PPP should also not be treated as waste by default. During double-spin PRP preparation, PPP may be removed to concentrate the platelet pellet, but it remains an autologous protein-rich plasma fraction. It can be used to adjust final PRP volume, dilute a highly concentrated pellet to a target dose, prepare controls in research settings, or generate plasma-derived matrices. ACD-based PRP often requires exogenous activation when clot formation or gelation is desired, commonly with calcium chloride or thrombin-related strategies [27].

Plasma-gel and matrix papers are best used here as technical plausibility evidence, not as proof of musculoskeletal clinical superiority. Godoi, Lana, and colleagues described a PRP-HA cellular gel matrix in which ACD-collected blood undergoes two centrifugation steps, the upper PPP fraction is converted into plasma gel by heating to 70 °C for approximately 15 minutes, and the plasma gel is mixed with leukocyte-rich PRP and hyaluronic acid [28]. Lana et al. later described a platelet-rich plasma power-mix gel protocol rich in growth factors and fibrin [29]. Nakamura et al. verified thermally prepared PPP plasma gel as a carrier matrix in vitro [30].

These papers are predominantly laboratory, matrix-development, or non-orthopedic sources. They show that the same blood draw can be conceptualized as a modular preparation platform: PRP, PPP, buffy coat, activated gel, or matrix components can be selected according to clinical intent. They do not establish that plasma gel improves every musculoskeletal indication.

For orthopedic readers, the practical message is not that plasma gel should replace PRP. It is that one preparation episode can generate multiple auditable autologous fractions: concentrated platelets, PPP volume/proteins, buffy-coat cells, or a gelled retention matrix. Keeping these fractions separate or deliberately recombining them is an advantage over poorly described one-syringe products.

This customizability also creates responsibility. A manuscript should not merely state that PRP was prepared from ACD tubes. It should state whether the buffy coat was avoided, skimmed, or intentionally collected; whether the platelet pellet was resuspended in PPP; whether PPP was discarded, reinjected, gelled, or used to adjust volume; and whether the final product was activated. These choices determine whether two clinicians using the same first and second spin are actually delivering the same biologic product.

Reporting standards and cost-aware translation

The main scientific risk of manual PRP is not that it is low cost; it is that low-cost techniques can be underreported. The minimum reportable dataset should include tube manufacturer and catalog number, ACD formulation, nominal and actual fill volume, total blood volume, number of tubes, centrifuge model, rotor type, rotor radius, RCF rather than RPM alone, spin duration, temperature, brake setting, delay from venipuncture to processing, open/closed status, number of transfers, aspiration layer, buffy-coat strategy, PPP destination, final volume, platelet concentration, total delivered platelet dose, leukocyte subsets when feasible, erythrocyte contamination, activation status, sterility controls, material cost, staff time, equipment requirements, pooling, and intended indication [11,12,31,32,33].

Cost reporting should be dose-normalized. A protocol intended to improve access should state disposable material cost, number of tubes, number of spins, staff time, equipment requirements, sterility controls, and whether the final dose requires pooling of multiple tubes. This avoids the false comparison between a single inexpensive tube that delivers a low platelet dose and a commercial kit that processes a larger blood volume. The fair comparison is cost per billion delivered platelets, cost per usable PRP milliliter, and cost per clinically intended treatment episode.

When framed this way, manual ACD-tube preparation may remain operationally feasible while reducing disposable material costs, even when repeated processing is required. If the target clinical dose is several billion platelets, one tube may be insufficient. Processing several low-cost ACD tubes may nevertheless remain less expensive than proprietary disposables, while preserving the ability to customize leukocyte content and retain PPP for gel or matrix applications. This is especially important for resource-constrained environments, sports medicine practices with high procedure volume, and health systems seeking reproducible biologics without dependence on a single commercial platform.

Safety reporting should be similarly pragmatic. Protocols should document sterile field, open/closed status, transfers, stopper punctures, time to injection, filtration, activation, post-injection flare, and any PPP-gel heating, cooling, or mixing sequence.

A low-cost manual protocol must therefore satisfy three conditions. First, it must produce a biologically plausible product with a reported dose and composition. Second, it must be reproducible enough that another clinician can repeat the preparation with comparable output. Third, it must lower access barriers without shifting hidden costs to contamination risk, operator variability, inadequate dose, or irreproducible layer selection. This is a stricter claim than simply saying that ACD tubes are cheap.

Table 2. Practical translation of ACD-tube PRP into dose-aware, cost-aware, customizable musculoskeletal practice.

Practical question	Best-supported interpretation	Evidence calibration	Operational implication
Which open ACD protocol is the current candidate?	600 × g/5 min followed by 900 × g/15 min had the highest open-protocol yield in Machado 2019.	Single study with internal protocol-numbering inconsistency; best available candidate, not established standard.	Use only with explicit citation, local validation, RCF reporting, and final cell counts.
Which closed low-cost protocol is supported?	Turn Down-Turn Up reached 4.17-fold enrichment and 73.6% recovery in a closed tube workflow.	Promising technical evidence; comparison with 600 + 900 × g was not statistically conclusive.	Useful where contamination control and low material cost are priorities.
How should dose be interpreted?	Absolute deliverable platelets are more clinically interpretable than fold enrichment alone.	Strongest clinical dose data come from knee osteoarthritis; other indications remain extrapolative.	Report baseline count, processed blood volume, final concentration, injected volume, and calculated dose.
When are multiple tubes justified?	One 8.5 mL tube at 200,000/µL contains about 1.7 billion platelets before losses.	Higher-dose targets may require repeated low-cost processing or larger blood volume.	Report pooling, sterility, staff time, and cost per billion delivered platelets.
Should buffy coat be collected?	Avoidance generally fits intra-articular OA; inclusion can be considered for selected soft-tissue rationales.	Mechanistic and indication-dependent; not a universal rule.	State whether buffy coat was avoided, skimmed, or intentionally collected.
What about PPP and plasma gel?	PPP can adjust volume or be converted into plasma-derived matrix/gel.	Mostly laboratory, dermatologic, aesthetic, or non-orthopedic evidence; supports plausibility and modularity, not broad clinical superiority.	Report PPP destination, activation/heating protocol, mixing sequence, and intended role.

Table 3. Minimum reporting checklist for manual ACD-tube PRP preparation.

Checklist domain	Minimum items to report	Why it matters
Tube and anticoagulant	Manufacturer, catalog number, ACD formulation, nominal volume, actual fill volume, number of tubes, total blood volume.	Tube geometry and anticoagulant concentration affect sedimentation, platelet behavior, and comparability.
Centrifuge physics	Centrifuge model, rotor type, rotor radius, RCF for each spin, duration, temperature, brake setting, and time from venipuncture to processing.	RPM alone cannot be transferred across rotors; RCF and timing define the separation process.
System sterility	Open, closed, or semi-closed status; number of transfers; needle passes through stoppers; sterile field; final time to injection.	Low-cost workflows must not hide contamination risk or operator-dependent handling.
Layer harvest	Aspirated layer, aspiration depth, buffy-coat strategy, pellet resuspension method, PPP removed or retained.	Layer selection directly changes platelet, leukocyte, and erythrocyte composition.
Final product composition	Final volume, platelet concentration, administered platelet dose, leukocyte total/differential when feasible, RBC contamination, activation status.	Clinical interpretation requires the actual biologic product, not only the preparation recipe.
Dose calculation	Baseline platelet count × blood volume; expected recovery; final concentration × injected volume; losses and pooling noted.	Separates concentration, recovery, and administered dose.
PPP and matrix use	PPP discarded, reinjected, used for dilution, heated into plasma gel, mixed with PRP/HA, or used as research control.	Makes modular workflows auditable and prevents unsupported claims of clinical benefit.
Cost and feasibility	Disposable cost, equipment requirement, staff time, number of spins, repeated processing, cost per billion delivered platelets.	Allows fair dose-normalized comparison with commercial kits.
Clinical intent	Target indication, intended leukocyte phenotype, target volume, target platelet dose, image guidance if applicable.	Links preparation choices to the musculoskeletal use case.

Synthesis and clinical implications

The most defensible synthesis is that ACD-tube PRP is a preparation platform, not a single recipe. Within the limited ACD technical literature, double-spin protocols are reasonable candidates when the goal is platelet enrichment. The 600 × g for five minutes followed by 900 × g for 15 minutes open regimen is best described as the highest-yield open ACD-A candidate in one internally inconsistent study, not as an established standard. The closed Turn Down-Turn Up method is a separate low-cost candidate with high recovery. Both should be reported alongside absolute dose and product composition.

Clinically, the protocol should start with the target product. For intra-articular osteoarthritis, an erythrocyte-poor and generally leukocyte-poor product with a reported absolute platelet dose is the most coherent default. For chronic tendinopathy or soft-tissue repair, buffy-coat inclusion may be considered when mononuclear-cell biology is part of the rationale, but this should be deliberate and documented. For surgical or matrix-based applications, PPP and activated plasma gel may be relevant as retention or carrier strategies, while clinical benefit remains indication-specific and incompletely established. No evidence currently supports a universal platelet-dose threshold for tendinopathy, ligament injury, muscle injury, or surgical augmentation.

For research, the implications are straightforward. Studies should stop reporting only centrifuge RPM, fold increase, or commercial kit name. They should report the entire process from patient blood to delivered product, including the cost and dose consequences of repeated tubes or pooled runs. A concise narrative review can preserve depth by focusing on these high-yield variables rather than trying to summarize every PRP trial.

A practical ACD-tube workflow can be summarized in five steps. First, define the target product before drawing blood: leukocyte-poor intra-articular PRP, leukocyte-rich soft-tissue PRP, PRP plus PPP dilution, or matrix-oriented PRP. Second, estimate the platelet dose required from baseline platelet count, tube volume, expected recovery, and target injected volume. Third, select a double-spin protocol and document RCF, time, rotor, tube geometry, and temperature. Fourth, control aspiration depth and buffy coat handling. Fifth, verify final volume and cell counts whenever feasible.

This workflow also helps reviewers evaluate manuscripts. Studies that omit dose, product composition, or aspiration layer may be clinically interesting but difficult to reproduce. Transparent ACD-tube reporting protects both positive and negative findings from overinterpretation.

Limitations

This review is narrative rather than systematic, so study selection remains vulnerable to author judgment despite a structured scope and search statement. The primary ACD-tube literature is small, mostly technical, and not powered for clinical outcomes. Several studies use different tube sizes, anticoagulant formulations, centrifuges, rotors, and aspiration methods, limiting direct comparison. No primary ACD-tube study has defined a centrifugal force threshold for subclinical platelet activation using markers such as P-selectin, PF4, beta-thromboglobulin, or thromboxane B2.

A further limitation is concentration of the source literature. Several technical, dose, and matrix claims trace to a small number of overlapping author groups, reducing the independence of the evidence base. The central 600 + 900 × g open-protocol candidate rests on one Machado 2019 dataset that contains an internal protocol-numbering inconsistency and lacks definitive independent replication. The domain-based appraisal used here improves transparency but is not a validated risk-of-bias instrument for PRP preparation studies.

The dose discussion also relies heavily on knee osteoarthritis literature and should not be extrapolated uncritically to tendons, ligaments, muscle injury, wound applications, or surgical augmentation. Likewise, plasma gel and PRP matrix papers support biologic plausibility and technical modularity, but many are laboratory, dermatologic, aesthetic, or non-orthopedic sources and do not establish clinical superiority for every musculoskeletal indication. Finally, lower disposable material cost is not the same as formal cost-utility evidence; future studies should include dose-normalized cost and outcome data.

Conclusion

Manual ACD-tube PRP can fit orthopedic and musculoskeletal practice when it is treated as an accessible, reportable, and customizable biologic platform. The limited current technical literature favors double-spin preparation, careful aspiration strategy, explicit dose reporting, and indication-specific decisions about buffy-coat inclusion, but the main open-protocol candidate should be interpreted as best available rather than established. Low-cost ACD workflows may remain operationally feasible with lower disposable material costs even when repeated tubes or pooled runs are needed to reach clinically appropriate platelet doses.

The next step for the field is not another unreported PRP recipe. It is protocol-level transparency: tube, centrifuge, force, time, layer, buffy coat, PPP use, final volume, absolute dose, activation, leukocyte profile, erythrocyte contamination, and cost. With those variables reported, independent validation studies can compare manual ACD-tube preparation fairly with commercial systems and translate it more responsibly into musculoskeletal care.

Author track declaration

This manuscript is prepared for the OSCRSJ Narrative Review standard track. Senior-author details and complete author information are provided only on the separate title page to preserve blinded peer review.

AI tools used

OpenAI ChatGPT/Codex was used to assist with condensation from a longer manuscript, language editing, formatting, consistency checks, and preparation of submission files. The authors reviewed and verified the scientific content, citations, and final wording.

Conflict of interest disclosure

Author-identifying conflict-of-interest disclosures are provided on the separate title page and in the submission portal, consistent with the journal's blinded peer-review process.

Declarations

Funding: No external funding was received for this work.

Conflicts of Interest: The authors declare no conflicts of interest. The full disclosure of potential conflicts of interest is provided with the online version of this article.

Patient Consent: This report does not involve identifiable patient data; patient consent was not applicable.

IRB / Ethics Approval: Institutional Review Board approval was not required for this report per institutional policy.

Data Availability: All data are included in the manuscript

Author Contributions (CRediT): V.F.P. — Conception and study design; project coordination; literature search; evidence acquisition; critical appraisal of the literature; data interpretation; manuscript drafting; manuscript writing; critical revision; supervision; final approval of the submitted version.; G.H.F. — Study design; literature search; evidence acquisition; critical appraisal of the literature; data interpretation; manuscript writing; critical revision; final approval of the submitted version.; R.D.C.S. — Literature search; evidence acquisition; manuscript editing; critical revision; final approval of the submitted version.; N.A.F.N. — Literature search; evidence acquisition; manuscript editing; critical revision; final approval of the submitted version.; G.S.B. — Literature search; evidence acquisition; manuscript editing; critical revision; final approval of the submitted version.; F.B.R. — Senior scientific supervision; critical revision of the manuscript; interpretation of the evidence; final approval of the submitted version.

Use of Generative AI: AI Tools Used: OpenAI ChatGPT/Codex was used to assist with condensation from a longer manuscript, language editing, formatting, consistency checks, and preparation of submission files. The authors reviewed and verified the scientific content, citations, and final wording.

References

1. Dhurat R, Sukesh M. Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. J Cutan Aesthet Surg. 2014;7(4):189-197. doi:10.4103/0974-2077.150734.
2. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med. 2006;34(11):1774-1778. doi:10.1177/0363546506288850.
3. Pachito DV, Bagattini AM, de Almeida AM, Mendrone-Junior A, Riera R. Technical procedures for preparation and administration of platelet-rich plasma and related products: a scoping review. Front Cell Dev Biol. 2020;8:598816. doi:10.3389/fcell.2020.598816.
4. Boswell SG, Cole BJ, Sundman EA, Karas V, Fortier LA. Platelet-rich plasma: a milieu of bioactive factors. Arthroscopy. 2012;28(3):429-439. doi:10.1016/j.arthro.2011.10.018.
5. Filardo G, Kon E, Buda R, et al. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surg Sports Traumatol Arthrosc. 2011;19(4):528-535. doi:10.1007/s00167-010-1238-6.
6. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. Am J Sports Med. 2013;41(2):356-364. doi:10.1177/0363546512471299.
7. Di Martino A, Di Matteo B, Papio T, et al. Platelet-rich plasma versus hyaluronic acid injections for the treatment of knee osteoarthritis: results at 5 years of a double-blind, randomized controlled trial. Am J Sports Med. 2019;47(2):347-354. doi:10.1177/0363546518814532.
8. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol. 2009;27(3):158-167. doi:10.1016/j.tibtech.2008.11.009.
9. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. Arthroscopy. 2012;28(7):998-1009. doi:10.1016/j.arthro.2012.04.148.
10. Mautner K, Malanga GA, Smith J, et al. A call for a standard classification system for future biologic research: the rationale for new PRP nomenclature. PM R. 2015;7(4 Suppl):S53-S59. doi:10.1016/j.pmrj.2015.02.005.
11. Murray IR, Geeslin AG, Goudie EB, Petrigliano FA, LaPrade RF. Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO): platelet-rich plasma and mesenchymal stem cells. J Bone Joint Surg Am. 2017;99(10):809-819. doi:10.2106/JBJS.16.00793.
12. Chahla J, Cinque ME, Piuzzi NS, et al. A call for standardization in platelet-rich plasma preparation protocols and composition reporting: a systematic review of the clinical orthopaedic literature. J Bone Joint Surg Am. 2017;99(20):1769-1779. doi:10.2106/JBJS.16.01374.
13. Dashore S, Chouhan K, Nanda S, Sharma A. Preparation of platelet-rich plasma: National IADVL PRP Taskforce recommendations. Indian Dermatol Online J. 2021;12(Suppl 1):S12-S23. doi:10.4103/idoj.idoj_269_21.
14. do Amaral RJFC, da Silva NP, Haddad NF, et al. Platelet-rich plasma obtained with different anticoagulants and their effect on platelet numbers and mesenchymal stromal cells behavior in vitro. Stem Cells Int. 2016;2016:7414036. doi:10.1155/2016/7414036.
15. Machado ES, Leite R, dos Santos CC, et al. Turn down - turn up: a simple and low-cost protocol for preparing platelet-rich plasma. Clinics (Sao Paulo). 2019;74:e1132. doi:10.6061/clinics/2019/e1132.
16. Baethge C, Goldbeck-Wood S, Mertens S. SANRA-a scale for the quality assessment of narrative review articles. Res Integr Peer Rev. 2019;4:5. doi:10.1186/s41073-019-0064-8.
17. Giraldo CE, Alvarez ME, Carmona JU. Effects of sodium citrate and acid citrate dextrose solutions on cell counts and growth factor release from equine pure-platelet rich plasma and pure-platelet rich gel. BMC Vet Res. 2015;11:60. doi:10.1186/s12917-015-0370-4.
18. Machado ES, Soares FP, Yamaguchi RS, et al. A simple double-spin closed method for preparing platelet-rich plasma. Cureus. 2022;14:e20899. doi:10.7759/cureus.20899.
19. Gupta V, Parihar AS, Pathak M, Sharma VK. Comparison of platelet-rich plasma prepared using two methods: manual double spin method versus a commercially available automated device. Indian Dermatol Online J. 2020;11(4):575-579. doi:10.4103/idoj.IDOJ_653_19.
20. Mota ML, Barreto RB, Leite BR, Cavalcante BCCD. Development of a device to obtain platelet-rich plasma using conventional materials. Rev Bras Ortop (Sao Paulo). 2021;57(2):289-294. doi:10.1055/s-0040-1721835.
21. Harrison TE, Bowler J, Cheng CI, Reeves KD. Optimizing platelet-rich plasma: spin time and sample source. Bioengineering (Basel). 2023;10(11):1270. doi:10.3390/bioengineering10111270.
22. Berrigan WA, Bailowitz Z, Park A, Reddy A, Liu R, Lansdown D. A greater platelet dose may yield better clinical outcomes for platelet-rich plasma in the treatment of knee osteoarthritis: a systematic review. Arthroscopy. 2025;41(3):809-817.e2. doi:10.1016/j.arthro.2024.03.018.
23. Berrigan W, Tao F, Kopcow J, et al. The effect of platelet dose on outcomes after platelet rich plasma injections for musculoskeletal conditions: a systematic review and meta-analysis. Curr Rev Musculoskelet Med. 2024;17(12):570-588. doi:10.1007/s12178-024-09922-x.
24. Bansal H, Leon J, Pont JL, et al. Platelet-rich plasma (PRP) in osteoarthritis (OA) knee: correct dose critical for long term clinical efficacy. Sci Rep. 2021;11(1):3971. doi:10.1038/s41598-021-83025-2.
25. Sundman EA, Cole BJ, Karas V, et al. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. Am J Sports Med. 2014;42(1):35-41. doi:10.1177/0363546513507766.
26. Lana JF, Huber SC, Purita J, et al. Leukocyte-rich PRP versus leukocyte-poor PRP: the role of monocyte/macrophage function in the healing cascade. J Clin Orthop Trauma. 2019;10(Suppl 1):S7-S12. doi:10.1016/j.jcot.2019.05.008.

27. Patel H, Pundkar A, Shrivastava S, Chandanwale R, Jaiswal AM. A comprehensive review on platelet-rich plasma activation. Cureus. 2023;15(11):e48943. doi:10.7759/cureus.48943.
28. Godoi TTF, Rodrigues BL, Huber SC, et al. Platelet-rich plasma gel matrix (PRP-GM): description of a new technique. Bioengineering (Basel). 2022;9(12):817. doi:10.3390/bioengineering9120817.
29. Lana JF, Purita J, Everts PA, et al. Platelet-rich plasma power-mix gel (ppm)-an orthobiologic optimization protocol rich in growth factors and fibrin. Gels. 2023;9(7):553. doi:10.3390/gels9070553.
30. Nakamura M, Masuki H, Kawabata H, et al. Plasma gel made of platelet-poor plasma: in vitro verification as a carrier of polyphosphate. Biomedicines. 2023;11(11):2871. doi:10.3390/biomedicines11112871.
31. Perez AGM, Lichy R, Lana JFSD, Rodrigues AA, Luzo ACM, Belangero WD, Santana MHA. Prediction and modulation of platelet recovery by discontinuous centrifugation of whole blood for the preparation of pure platelet-rich plasma. Biores Open Access. 2013;2(4):307-314. doi:10.1089/biores.2013.0015.
32. Perez AGM, Lana JF, Rodrigues AA, Luzo ACM, Belangero WD, Santana MHA. Relevant aspects of centrifugation step in the preparation of platelet-rich plasma. ISRN Hematol. 2014;2014:176060. doi:10.1155/2014/176060.
33. Lana JFSD, Purita J, Paulus C, et al. Contributions for classification of platelet rich plasma - proposal of a new classification: MARSPILL. Regen Med. 2017;12(5):565-574. doi:10.2217/rme-2017-0042.

Cite this article as: Pacheco VF, Fornasari GH, Serafim RDC, Neta NAF, Barbiero GS, Ribeiro FB. manual acd-tube prp for musculoskeletal care: accessible protocols, dose, and customization. *OSCRSJ*. 2026;1(1):e0003. https://doi.org/10.XXXXX/oscrsj.2026.0003