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TECHNICAL NOTE

MIBlood-EV: Minimal information to enhance the quality and reproducibility of blood extracellular vesicle research

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Abstract
Blood is the most commonly used body fluid for extracellular vesicle (EV) research. The composition of a blood sample and its derivatives (i.e., plasma and serum) are not only donor-dependent but also influenced by collection and preparation protocols. Since there are hundreds of pre-analytical protocols and over forty variables, the development of standard operating procedures for EV research is very challenging. To improve the reproducibility of blood EV research, the International Society for Extracellular Vesicles (ISEV) Blood EV Task Force proposes standardized reporting of (i) the applied blood collection and preparation protocol and (ii) the quality of the prepared plasma and serum samples. Gathering detailed information will provide insight into the performance of the protocols and more effectively identify potential confounders in the prepared plasma and serum samples. To collect this information, the ISEV Blood EV Task Force created the Minimal Information for Blood EV research (MIBlood-EV), a tool to record and report information about pre-analytical protocols used for plasma and serum preparation as well as assays used to assess the quality of these preparations. This tool does not require modifications of established local pre-analytical protocols and can be easily implemented to enhance existing databases thereby enabling evidence-based optimization of pre-analytical protocols through meta-analysis. Taken together, insight into the quality of prepared plasma and serum samples will (i) improve the quality of biobanks for EV research, (ii) guide the exchange of plasma and serum samples between biobanks and laboratories, (iii) facilitate inter-laboratory comparative EV studies, and (iv) improve the peer review process.

KEYWORDS
biomarker, blood, extracellular vesicles, liquid biopsy, quality control, reproducibility, standardization

1 | INTRODUCTION

Blood is the most used biofluid for liquid biopsy research and is a critical source of easily accessible analytes for diagnostic testing (Royo et al., 2020). Blood-derived plasma and serum are the most commonly studied biofluids for extracellular vesicle (EV) research. Blood is a complex fluid rich in soluble macromolecules (including lipoproteins), cells (intact and fragmented), and non-vesicular nucleic acids, that can strongly overlap with EVs in physical properties (i.e., size, density) and composition. Due to their similarities, it is very challenging to separate EVs from non-EV particles using currently available isolation methods such as ultracentrifugation, size exclusion chromatography, and density gradient ultracentrifugation (Théry et al., 2018). Resultingly, the presence of heterogeneous non-EV macromolecules can confound downstream EV analysis. For example, the study of EV-associated miRNA can be affected by the presence of other miRNA carriers such as soluble proteins, platelets, cell remnants and lipoproteins co-isolated with EVs.

While obtaining a pure EV preparation remains an unachieved goal, there is a growing awareness that the initial quality of the source biospecimen (i.e., whole blood, plasma, or serum) is a critical determinant for the success and reproducibility of downstream studies of EVs. Importantly, pre-analytical protocols used for blood collection, processing, and storage strongly influence the presence of confounders in plasma and serum (Dhondt et al., 2023, Karimi et al., 2022, Małys et al., 2023). Noteworthy, several studies have reported a significant difference in the molecular composition between plasma- and serum-derived EV preparations from blood of the same donors (Dhondt et al., 2023, Muraoka et al., 2022, Zhang et al., 2022). Over the last decade, the efforts of the International Society for Extracellular Vesicles (ISEV), including its collaboration with the International Society on Thrombosis and Haemostasis (ISTH), and the International Society for Advancement of Cytometry (ISAC), have led to the publication of several position papers and evidence-based guidelines for blood EV research (Table 1) (Clayton et al., 2019, Lacroix et al., 2013, Lötvall et al., 2014, Théry et al., 2018, Witwer et al., 2013). In practice, however, most laboratories still use "in-house" protocols, which are often contingent on the availability of local infrastructure and downstream applications. The heterogeneity of pre-analytical protocols is illustrated by a recent survey amongst members of the GEIVEX, the Spanish EV society, which indicated variability in pre-analytical protocols of biospecimens as high as 94%. Notably, none of the seventy respondents within the survey used the same protocol for blood collection, plasma or serum preparation, and storage (López-Guerrero et al., 2023). Thus, from the perspective of a single downstream application, pre-analytical protocols are inconsistent. A summary of nineteen recent

TABLE 1 Existing guidance for the preparation and reporting of blood-derived extracellular vesicle biospecimens.

Manuscript	Journal	Publication year	Take-aways
Witwer et al. (2013)	Journal of Extracellular Vesicles	2013	Need for standardization in specimen handling, normative controls, and analysis techniques
Coumans et al. (2017)	Circulation Research	2017	Considerations and recommendations from sample collection to isolation
Clayton et al. (2018)	Journal of Extracellular Vesicles	2018	More effort is needed to standardize pre-analytical issues and benchmark isolation methods
Clayton et al. (2019)	Journal of Extracellular Vesicles	2019	A roadmap for rigorous and reproducible blood-derived EV research

publications on EV-associated miRNAs showed that in four studies (20%) neither blood collection nor plasma preparation protocols were described, whereas in the other studies, the pre-analytical protocols were incomparable (Bracht et al., 2023). In this light, standard reporting guidelines are known to promote research consistency, and, consequentially, encourage reproducibility and rigorous study design. While standardization of pre-analytical protocols for blood collection, processing, and storage is still an unmet and challenging goal, the ISEV Blood Task Force proposed in 2019 a roadmap to improve the rigour and reproducibility of blood EV research, which includes the need to evaluate the quality of plasma and serum.

In response to this identified need, we developed the Minimal Information for Blood EV research, namely MIBlood-EV, a tool that enables the transparent reporting of not only pre-analytical parameters for blood collection, processing, and storage but also the quality of prepared plasma and serum; while possibly applicable to laboratory animals (e.g., rodents, large animals) this reporting tool was developed for use with human sampling considerations. We used results from two surveys sent to ISEV members and members of the Blood EV Task Force to incorporate quality indicators and quantitative methods to assess the presence of confounders. The MIBlood-EV reporting tool aims to (i) improve the quality of biobanks for EV research, (ii) allow the exchange of plasma and serum samples between biobanks and laboratories, (iii) facilitate inter-laboratory comparative EV studies, (iv) improve the peer review process, and, finally (v) facilitate implementation without the modification of established local pre-analytical protocols.

The initial version of the MIBlood-EV was developed as a user-friendly editable document, but it is our ambition to integrate it into the centralized database for reporting EV research studies such as EV-TRACK (Van Deun et al., 2017). Thus, rather than telling researchers "what to do," we offer an alternative solution by providing a tool for transparent reporting. Incorporation of the MIBlood-EV into a centralized database will allow retrospective analysis of results and the subsequent development of evidence-based standard operating procedures (Lehmann et al., 2012). Doing so, we will collectively continue to improve the reproducibility of blood EV research which, in turn, will ultimately accelerate multi-institutional research studies and the translation of discoveries into the clinic.

2 | METHODS

2.1 | Origin and development of surveys

A group of five members of the ISEV Blood Task Force (F.L., D.G., M.L., B.L., and R.N.) reviewed existing articles containing recommendations for blood-derived EV reporting. Following the guidelines on the development of reporting standards and processes recommended by existing literature (Table 1), the authors created a comprehensive list of criteria and metrics used to assess the quality of biospecimens for research. From this list, the expert panel modified and removed items on the consensus basis from two online questionnaires targeting two distinct groups: (i) members of the ISEV Blood Task Force with more than five years of experience in blood EV research ($n = 16$ respondents), and (ii) junior and senior ISEV members ($n = 176$ respondents). The participants were asked to identify what they consider as critical confounders in plasma and serum samples (Figure S1), what qualitative and quantitative methods they use to assess the presence of cofounders (Figure S2), and which strategies should be used to report quality control (QC) results (Figures S3 and S4). Information collected through surveys is available in Supplementary Materials. Responses to the survey served as a framework for the development of the MIBlood-EV.

2.2 | Development of the MIBlood-EV tool

Following the guidelines on the development of reporting standards suggested by the "Enhancing the Quality and Transparency Of Health Research Network," our group created a comprehensive list of key guideline items (Ogrinc et al., 2016). Following

iterative rounds of discussion and survey data incorporation, we curated a consensus list of elements to be present in the final reporting document. On the basis of this process, additional changes and clarifications were made by other members of the ISEV Blood Task Force until we reached a consensus reporting tool suitable for widespread adoption by the blood EV community. Through this, the MIBlood-EV was developed, which can be completed and attached to all scientific manuscripts reporting the use of blood or its derivatives (i.e., plasma and serum) for EV research.

3 | RESULTS

3.1 | Considerations and recommendations for completion of MIBlood-EV version 1.0

The MIBlood-EV is a tool that enables transparent reporting of pre-analytical protocols and quality of plasma and serum prepared for EV research. The MIBlood-EV version 1.0 includes 27 items that encompass the following components: (i) General study information (items 1.0–1.9), (ii) Blood collection and processing (items 2.0–2.23), and (iii) Plasma/serum quality control (items 3.0–3.27, Supplementary File 1). Since quality controls are the key to monitoring the quality of the prepared plasma and serum samples, we provide brief background information for the three common confounders (i.e., haemolysis, platelets and lipoproteins) and highlight methodological guidelines for their qualitative and quantitative assessment.

We recognize that some information requested in the MIBlood-EV may be difficult or impossible to retrieve. For instance, the number of centrifugations used to prepare plasma is not always reported with samples obtained from biorepositories or other external sources. As such, we recommend researchers provide as many methodological details as possible and clearly state when the information is not available using the abbreviation N/A.

We also recognize that QC of plasma and serum samples using quantitative analysis is not always feasible, for example, when large specimen cohorts are used, or when limited sample volume is available such as from paediatric patients, or when limited access to required equipment and funding resources. Quantitative analyses of haemolysis and lipoproteins are not routinely performed by the majority of non-clinical laboratories. Therefore, we recommend visual inspection of haemolysis and lipoprotein content as minimal information to report; standardized reference palettes can easily facilitate this. We invite the community to contribute to this "Reproducibility Initiative" and conduct quantitative analysis if the equipment and expertise are available. It becomes even more essential and relevant during methodological and benchmark studies. In this document, we describe the most commonly used methods to quantify haemolysis and lipoprotein concentrations. Quantitative analysis of platelet contamination is more straightforward as it can be done by routine haematology analyzers and by flow cytometry (conventional and advanced).

For both qualitative and quantitative QC of large series of samples, we recommend reporting data for a representative number of samples. When samples are obtained from a collaborator or a biobank with limited information on protocols used for blood collection and handling, a more thorough quantitative analysis is optimal. Within this context, measurements should be reported as range or median with 95% confidence interval and the number of samples tested. The final sample sizes should be stated and justified. Sample exclusion criteria (if utilized) should be clearly provided.

3.2 | Content of the MIBlood-EV v1.0

3.2.1 | Study Information (1.0-1.9)

Along with the information traditionally presented within a study (i.e., title, corresponding author, affiliation/institution, and time period of the study; items 1.0-1.3), authors should report the number of samples used in the study (item 1.4), cargo of interest (item 1.5), type(s) of biospecimens (item 1.6), as well as the state (e.g., fresh or frozen), source, and age of biospecimens (item 1.7-1.9). This information allows the reader to more effectively understand the rationale for methodological decisions and subsequently appreciate the potential limitations of the resulting data.

3.2.2 | Blood Collection and Processing (2.0 – 2.23)

After providing general study information, the MIBlood-EV includes questions on the methods used for blood collection, processing, transportation and storage, aligning with the evidence-based guidelines established by Coumans et al. (2017)

Patient Fasting Status (2.1): Different lipoprotein populations share biophysical characteristics with EVs, making it challenging and labour-intensive to separate EVs from all lipoproteins with high recovery and high specificity (Vergauwen et al., 2021, Zhang et al., 2020). While some studies have highlighted that fasting potentially reduces the quantity of very-low-density lipoproteins and chylomicrons in the circulation (Nakajima et al., 2011), the impact of fasting on EV isolation has yet to be fully understood.

Additionally, the consequences of fasting on the concentration and molecular composition of EVs remain to be elucidated. To that end, the fasting status, if known, should be reported (item 2.0) with the length of fasting if applicable (item 2.1).

Phlebotomy Protocol (2.2 – 2.7): While standardized phlebotomy protocols are in place at most medical centres, the impact of specific protocols (i.e., tube order, site of collection, processing time) on blood EV research has yet to be fully elucidated. Pre-analytical parameters can influence the quality and composition of EVs harvested from blood. Closed collection systems (e.g., winged butterfly needle with vacuum blood collection tubes) are recommended by the World Health Organization and the Clinical Laboratory Standards Institute for safety reasons and prevention of contamination (Bekeris et al., 2005). Open collection systems (syringe combined with a needle) can be used, but there is a risk for cross-contamination between different additives contained in blood collection tubes. As such, research groups using blood collected with open systems should follow the Clinical Laboratory Standards Institute recommendations on the order of draw tubes (Institute, 2017). Needle size used for venipuncture is another variable that can affect downstream blood EV analysis. A small needle size (>21-gauge) can induce haemolysis, and a 21-gauge straight needle is usually recommended in phlebotomy protocols (Heyer et al., 2012). In the MIBlood-EV, investigators should state the anatomical access site (item 2.2) and needle diameter (item 2.3). Several anticoagulants (e.g., ethylenediaminetetraacetic acid, citrate, heparin) can be used to prepare plasma from whole blood. The anticoagulant should be compatible with the downstream applications, and anticoagulants may differentially affect EV concentrations and composition (Buntsma et al., 2022, Palviainen et al., 2020). Therefore, the volume of blood collected (item 2.4) and type of anticoagulant used (item 2.5) should be reported. The use of a clot activator with or without gel separator (item 2.6) and the clotting time (item 2.7) should be indicated for serum, along with the reported volume of blood collected (item 2.4) and the type of anticoagulant used (item 2.5).

Transportation, Separation, and Storage of Blood Derivatives (2.8 – 2.23): Post-collection processing variables can also affect EV heterogeneity and contamination levels (Linares et al., 2015, Momen-Heravi et al., 2012, Wisgrill et al., 2016). As such, variables such as time interval between collection and centrifugation (item 2.8), transport temperature (item 2.9), and transport conditions of the tubes (e.g., upright vertical or horizontal; item 2.10) should be stated. Processing details including centrifuge and rotor model/brand (item 2.11), bucket type (item 2.12), number of centrifugation cycles (item 2.13), centrifugation speeds (item 2.14/2.17), rotor brake (item 2.15/2.18), and centrifugation temperature (item 2.16/2.19) should be reported. Additional processing steps such as filtration through polycarbonate and polyester membrane can improve platelet removal for downstream applications (Bettin et al., 2022, Bracht et al., 2023). Any additional procedures applied to plasma and serum samples post-centrifugation should be stated (item 2.20). In addition, it is relevant to document the brand and type of storage tubes (item 2.21), storage temperature (item 2.22), and length of storage prior to utilization (item 2.23).

3.2.3 | Plasma/Serum Quality Control (3.0 – 3.27)

Lipoproteins, platelets, and fragmented platelets and erythrocytes (especially after a freeze-thaw cyle) can be major confounders of EV preparations (Supplementary Figure 1) (Aatonen et al., 2014, Yuana et al., 2014). The presence of some confounders can be determined qualitatively (e.g., visual inspection) or quantitatively (e.g., routine haematology analyzers, flow cytometry).

Freeze-Thaw Cycle Monitoring (3.0 – 3.2): Blood storage conditions, particularly the freeze-thaw cycling of plasma and serum samples, have downstream effects on EV properties such as concentration, size, and contaminant protein association (Gelibter et al., 2022). To ensure comprehensive reporting, the number of freeze-thaw cycles (item 3.0), thawing temperature (item 3.1), and thawing duration (item 3.2) should be documented.

Hemolysis (3.3 – 3.10): While best practices for phlebotomy and blood processing can help to minimize erythrocyte fragmentation, it is still important to monitor for the presence of haemolysis (item 3.3) and report the percentage of affected samples (item 3.4). Visual inspection is straightforward and can be routinely performed using a haemolysis reference palette (https://www.cdc.gov/ncezid/dvbd/stories/research-lab-diagnostics/hemolysis-palette.html). Quantitative methods using haematology analyzers and spectrophotometers (Gislefoss et al., 2021) can also be employed. Therefore, the analytical method used to assess haemolysis should be stated (item 3.5). For haematology analyzers, red blood cell counts (item 3.6) and instrument brand and type (item 3.7) should be stated. For spectrophotometry, haemoglobin concentrations (g/L) should be provided (item 3.8) with instrument specifications (item 3.9). Finally, it is important to state whether samples were included or excluded based on haemolysis, along with a clear justification for the decision (item 3.10).

Platelets (3.11 – 3.17): Resting platelets, which are discoid with a diameter of approximately 2–4 μm, pose a challenge for complete removal via centrifugation without impacting the concentration of EVs (Bettin et al., 2022, Małys et al., 2023). During a freeze-thaw cycle, platelets may fragment, and both platelets and platelet fragments can influence quantitative and functional EV assays (Kim et al., 2022, Yuana et al., 2015). Remaining platelets in plasma can be removed by filtration without affecting concentrations of EVs, although dedicated filters with small dead volumes are not yet commercially available. The concentration of residual platelets in plasma and serum is investigator and protocol-dependent. Therefore, the concentration of residual platelets in the prepared samples should be reported (Bettin et al., 2022). Haematology analyzers are routinely used to measure platelet concentrations but sometimes can fall below the lower limit of detection. Flow cytometry has been shown as a suitable alternative method to quantify concentrations of platelets (Bettin et al., 2022). Researchers should thus clearly state if the presence of

platelets was quantified (item 3.11). The methodology used to quantify platelet presence should be stated (item 3.12) along with the identity of the markers used (e.g., CD61 or CD41; item 3.13) and the concentration (item 3.14). If an automated blood analyzer is used, the technical information (i.e., brand and product name) and detection limit should be indicated (item 3.15), while the size and fluorescence ranges of detection for platelets should be provided in standardized units (nanometres and molecules of equivalent soluble fluorochrome) for flow cytometric techniques (item 3.16 and 3.17).

Lipoproteins (3.18 – 3.27): While fasting might potentially reduce the presence of larger lipoproteins such as very-low-density lipoproteins and chylomicrons in blood, it is often difficult to implement in clinical studies. In both fasting and non-fasting individuals, the presence of high concentrations of lipoproteins (lipemia) in prepared plasma and serum should be reported (item 3.18) (Sakai et al., 2003, Yuana et al., 2015). While clinical devices such as the Afinion Lipid Panel (Abbott) might be best suited for a broad assessment of lipoproteins, their limited availability to all researchers poses problems. There is nonetheless utility in using both quantitative (i.e., flow cytometry, ELISA) and semi-quantitative methods such as spectrophotometry (turbidimetry) (Hunsaker et al., 2019) or Western blot for these purposes as they can complement downstream assessment of EV preparations (Karimi et al., 2018). Investigators should indicate the method used to detect lipoproteins (item 3.19). For spectrophotometry, L-index is commonly used to report lipidaemia, therefore, L-index (item 3.20) and instrument type (item 3.21) should be indicated (Hunsaker et al., 2019). Given MISEV2018 recommends the analysis of at least one marker of apolipoproteins among ApoA1/2, ApoB-100/B-48 to assess lipoprotein abundance in EV samples, it may be appropriate to assess this in the blood derivatives as a means of determining percent reductions (Théry et al., 2018). For Western blot, apolipoprotein B (i.e., B-100/B-48) is a widely used lipoprotein marker because it is present in chylomicrons as well as high- intermediate- low- and very low-density lipoproteins, while apolipoprotein A can be used to measure the presence of high-density lipoproteins (Elovson et al., 1988, Feingold et al., 2000). For Western blot, we recommend reporting the marker(s) used (item 3.22) and providing full blot images to promote interpretation (item 3.23). Chylomicrons are the largest form of lipoproteins and can be detected by high-sensitivity flow cytometry (Botha et al., 2022). If such a method is used, the molecular marker(s) (item 3.24), stained particle concentration(s) (item 3.25), instrument specifics (item 3.26) and sensitivity range (item 3.27) should be reported.

4 | IMPLEMENTATION

The MIBlood-EV can facilitate interpretation and reproducibility of research findings and peer review of blood EV studies. The MIBlood-EV will be accessible on the ISEV website under Rigor & Standardization Subcommittee/Position Papers & Guidelines alongside the MIFlowCyt-EV (https://www.isev.org/rigor-standardization) (Botha et al., 2022). The original version (v1.0) is an editable pdf document that can be attached as Supplementary Material in manuscripts and referred to in sections of the Materials and Methods that describe blood specimens and characteristics. In addition, researchers are invited to upload the MIBlood-EV using the dedicated data repository https://forms.gle/S1Vs9Z2WifProMHM9. The file name should be as follows: MIBloodEV_First Author Last Name_First Author First Name_Year of Publication. Following the National Institute of Health Data Management and Sharing Policy, we recommend that MIBlood-EV be uploaded by the time of publication appears online or in print. The growing number of MIBlood-EV documents uploaded in the repository will enable the conduct meta-analyses and assess variability in pre-analytical conditions and sample quality across studies (Figure 1). Such work will facilitate the development of studies comparing the most commonly used protocols in blood collection, handling and storage and their impact on the quality of plasma and serum samples. Ultimately, access to this real-world information will serve as a framework for developing evidence-based guidelines and expert consensus statements (Dhondt et al., 2023). It is expected that a web-based version (v2.0) will be created and integrated into a centralized database for reporting EV-dedicated research protocols, such as EV-TRACK (Van Deun et al., 2017) or virtual biorepositories including exRNA atlas (Welsh et al., 2020) and the NanoFlow Repository (Murillo et al., 2019). This will further streamline and standardize reporting practices in the field.

The implementation of MIBlood-EV will be driven by the commitment of EV research groups to promote reproducibility by ensuring transparent reporting of blood sample quality and derivatives used in EV studies. To achieve widespread adoption, we recommend the endorsement of the MIBlood-EV by other international societies with long-standing collaboration with ISEV, including ISAC and ISTH. Furthermore, we encourage support from the International Society for Biological and Environmental Repositories, the global biobanking organization in charge of disseminating best practices for biorepositories, and the European Liquid Biopsy Society. By promoting the adoption of the MIBlood-EV, we can improve experimental rigor and reproducibility in EV research, accelerating the translation of discoveries into clinical practice.

5 | DISCUSSION

The MIBlood-EV was developed with the following priorities: the reporting tool should (i) be representative of a consensus across a broad cross-section of the EV research community, (ii) be translatable across a variety of applications by containing straightforward nomenclature and terminology, (iii) be organized such that it can serve as a tool for authors and peer reviewers,

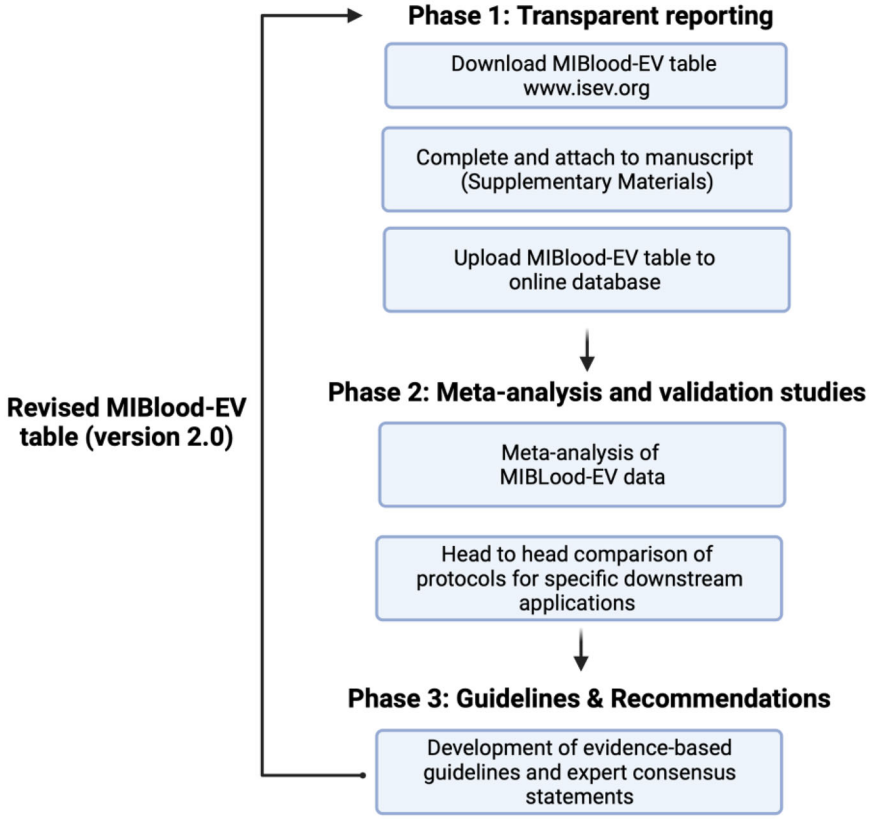

FIGURE 1 Proposed roadmap for MIBlood-EV implementation and utilization.

particularly when included in manuscript submission as a supplementary data file, and (iv) guide the complete and organized reporting of a study while emphasizing the tool should *not* serve as a point of enforcement for any particular reporting metric. A major strength of this tool is the rigor and transparency of its development by a diverse, multidisciplinary consortium of subject-matter experts.

While following the MIBlood-EV is essential for transparent reporting of blood sample quality and its derivatives, it does not negate the need for detailed reporting of the final quality of samples post-EV isolation. The recovery and purity of EVs is highly dependent on the EV separation and enrichment method used. As per MISEV2018 guidelines, it is recommended that researchers report all methodological details for enrichment of EVs as well as qualitative and quantitative characterization of their purity (e.g., presence of Apoliprotein B and albumin post-EV isolation).

It is important to note that adherence to a particular set of reporting guidelines should not be used to assign the quality or significance of a study. In this respect, the MIBlood-EV is designed to assist readers and reviewers in critically assessing studies, compliance with the MIBlood-EV should not be considered as an indicator of research quality. Rather, it should be seen as an effort to promote clear and transparent reporting of crucial steps. It is also important to note that the tool does not provide guidance on specific statistical and methodological decisions.

The ambition of the ISEV Blood Task Force is to provide, through a transparent and collaborative process, guidelines that iteratively and evidence-based will improve the quality of blood EV research. This Task Force believes that the proposed guidelines are flexible, balanced, and user-friendly, making them accessible to a broad range of scientists and clinicians and facilitating their widespread implementation in the EV research community.

AUTHOR CONTRIBUTIONS

Fabrice Lucien: Conceptualization; data curation; formal analysis; investigation; methodology; project administration-lead; resources; supervision; writing—original draft; writing—review and editing. **Dakota Gustafson**: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; supervision; writing—original draft; writing—review and editing. **Metka Lenassi**: Conceptualization; data curation; formal analysis; methodology; project administration; writing—original draft; writing—review and editing. **Bo Li**: Conceptualization; data curation; writing—review and editing. **Jacob J. Teske**: Resources; visualization-lead; writing—review and editing. **Eric Boilard**: Data curation; writing—review and editing. **Katharina Clemm von Hohenberg**: Data curation; writing—review and editing. **Juan Manual Falcón-Perez**: Data curation; writing—review and editing. **Alice Gualerzi**: Data curation; writing—review and editing. **Antonia Reale**: Data curation; writing—review and editing. **Jennifer C. Jones**: Data curation; writing—review and editing. **Cecilia Lässer**: Data

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CONFLICT OF INTEREST STATEMENT

J.C.J has collaborative research agreements with Beckman Coulter and Cytek Biosciences. R.P. received consulting fees from MetaGuideX. C.L. has collaborative research agreements with Astra Zeneca (Gothenburg, Sweden). E.B. received research funding from STRM Bio, Mitrix Bio, Veralox and Maresin Pharma. P.S. received funding support from the EV Ecosystem for Theranostic Platforms (Business Finland). K.W.W. has a collaborative research agreement with Ionis Pharmaceuticals; has served as an advisor to Exopharm, NeuroDex, Novadip, and ShiftBio; and performs ad hoc consulting on extracellular vesicles and RNA as Kenneth Witwer Consulting. All authors whose names are listed certify that they have no financial or non-financial conflicts of interest that would have had influence on the subject matter and materials nor recommendations contained within this manuscript.

DATA AVAILABILITY STATEMENT

The MIBlood-EV is presented as a versioned, editable, spreadsheet available for adaptation and inclusion in manuscripts. Anonymized survey results are included as a supplementary data source to further the transparency of the MIBlood-EV development.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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