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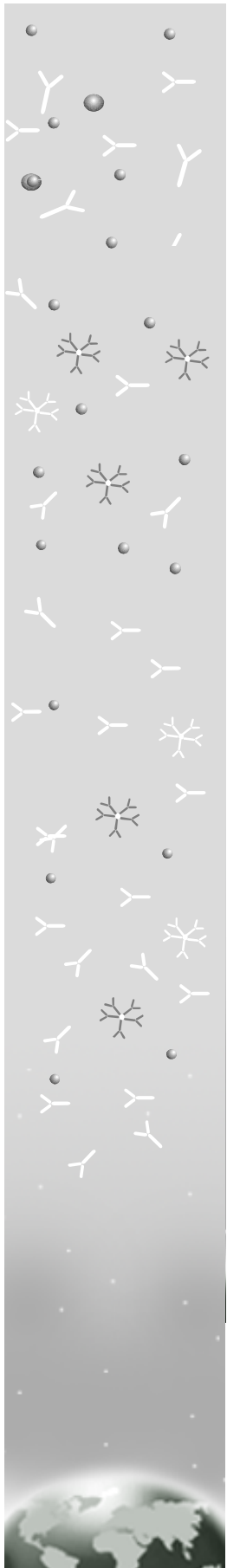
Preface

Tulip Group of companies believes in offering our valued customers the technical support and scientific information to keep updated with the latest international standards and trends in diagnostic testing.

Laboratory results play a pivotal role in providing the clinician the scientific data in diagnosing, monitoring and prophylaxis of deserving patients. Keeping in mind our valuable customers, Tulip Group will offer periodically a series of Tech Notes presented with a short, summarized overview pertaining to a specific technique/product/disease related information.

We hope that the Tech Notes will assist and benefit the laboratorians in enhancing the standards of reporting results thereby helping the clinician for better diagnosis and patient management.

Yours faithfully,
Tulip Diagnostics (P) Ltd.



Buffered 3.2% Tri-sodium Citrate the anticoagulant of choice for Routine Coagulation testing

General Background:

It is a known fact that pre-analytical variables play an important role in the final outcome of routine coagulation results. To improve precision and accuracy of haemostasis laboratory testing it is important to identify these variables and optimize them in order to ensure better diagnosis and monitoring of patients.

Pre-analytical variables pertinent to routine coagulation assays in a haemostasis laboratory can be classified into three major categories:

- Specimen Collection
- Specimen processing
- Specimen storage and transport

One of the most important pre-analytical variable associated with specimen collection is the anticoagulant used for obtaining plasma specimen. Tri-sodium Citrate is the anticoagulant of choice for coagulation studies because factor V and VIII are stable in citrate and integrity of other factors is also preserved.

Historically 3.8% (0.129M) citrate was used and is still being used as an anticoagulant for blood collection in coagulation studies. Though now internationally the trend is shifting towards the usage of 3.2% (0.109M) citrate as an anticoagulant for blood collection.

In the following pages we will briefly review the importance of 3.2% citrate vs. 3.8% citrate and its impact on PT and APTT results. Also the importance of buffered citrate will be discussed in this overview.

Discussion:

It is a debate whether 3.2% or 3.8% citrate concentration is ideal anticoagulant for coagulation studies. In an independent study conducted¹, the two citrate concentrations were compared with a responsive and less responsive PT and APTT reagents on five populations: healthy individuals, hospitalized patients not receiving anticoagulant therapy, patients receiving i.v. heparin therapy and patients receiving i.v. heparin and oral anticoagulant therapy.

Some very interesting but important facts were observed in this study.

1. The normal ranges with responsive PT and APTT reagents were significantly higher with 3.8% citrate concentration as compared to 3.2% citrate concentration.
2. Also the APTT results with patients receiving heparin therapy significant change in results were observed with responsive and less responsive APTT reagents with 3.8% citrate concentration as compared to 3.8% citrate concentration.

3. The INR values of patients under oral anticoagulant therapy also showed a significant change with responsive PT reagent and to a lesser extent with a less responsive PT reagent².

In all these observations the readings with 3.8% citrate concentration was on the higher side as compared to the readings observed with 3.2% citrate concentration when used as an anticoagulant for blood collection.

The results demonstrated that concentration of citrate used as an anticoagulant can vary clotting time probably because of the following reasons,

1. The amount of citrate present directly affects the calcium concentration of the reagent. Either 3.2% or 3.8% citrate binds all of the calcium present in the blood sample. But a higher citrate concentration (3.8%) binds more calcium from the reagent thereby leading to reduced concentration of calcium ions required for *in vitro* activation of clotting mechanism. This results in false prolongation of results especially with a responsive PT and APTT reagent.
2. Citrate concentration may also alter the haematocrit because citrate concentrations above 3.0% are hyperosmolar with blood. It was observed that as citrate concentration rises, a fairly uniform decrease in haematocrit occurs. Thus a higher citrate concentration (3.8%) would have a greater effect on clotting time especially on patient samples with abnormal haematocrits.
3. The most profound effect was noted in-patients receiving stable oral anticoagulant therapy where INR results differed significantly when a responsive PT reagent was used.

Effect of centrifugation on labile factors:

When anticoagulated blood is centrifuged for preparing PPP (platelet poor plasma) for routine coagulation assays, the centrifugation process results in release of CO₂. The end result being shift in pH, which has an adverse impact on results of clot based assays.

Thus usage of 3.2% buffered citrate (such as PROFACT available from Tulip Diagnostics (P) Ltd.) prevents the shift in pH due to the centrifugation process. As a result labile factor V and VIII are well preserved thereby improving accuracy and precision of results with clot based assays³.

Conclusion:

To conclude, the concentration of Tri-sodium Citrate is one of the key variables that has an impact on the end result of coagulation assays. In order to achieve precision, accuracy and reproducible results it is important to use,

1. Isosmolar 3.2% citrate concentration as recommended by the expert panel of International Society for Thrombosis and Haemostasis (ISTH).
2. Adequately buffered 3.2% citrate (PROFACT) to prevent pH related deterioration of factor V and VIII.

References and suggested readings:

1. Effect of 3.2% vs. 3.8% sodium citrate concentration on routine coagulation testing, D.M.Adcock, D.C. Kressin, R.A. Marlar, Journal of American Clinical Pathology, 1997, 107, 105-110.
2. Effect of Concentration of Tri-sodium Citrate anticoagulant on calculation of INR and ISI of thromboplastin, Duncan E.M., Thrombosis and Haemostasis, 1994, 72(1), 84-88.
3. Human Blood Coagulation, Haemostasis and Thrombosis, Edited by Rosemary Biggs, 1972, Blackwell Scientific Publications.