A Novel 2-Part System for the Culture of Bone Marrow Specimens for Cytogenetics Analysis

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Application Notes

- Significantly increases the number of mitotic figures
- Substantially improves chromosome morphology
- Minimizes specimen loss during shipping
- Improves specimen quality when stored for longer periods of time (up to 5 days)
- Prevents heparin toxicity

Introduction

The successful culture of bone marrow specimens is one of the most difficult challenges that cytogenetics labs experience. Two significant differences between bone marrow and other specimen types are that they are frequently smaller in size and more easily damaged in transport. These observations led to the realization that improvements in growth and morphology through medium optimization are limited, and true advancements would only be possible by improving *both* the transport and culture of specimens. Based upon this perspective, a novel two-part system for the improved transport, recovery and culture of bone marrow specimens for routine cytogenetic studies was developed.

The first part of the system focuses on transport of specimens to the laboratory. Bone Marrow Transfer Solution (BMTS; Product Code B 6426), a balanced nutrient mixture designed to maintain cells for up to five days prior to culture, was developed to improve transport. This product prevents the deterioration of low volume specimens in two key ways. It provides a liquid matrix that protects specimens from drying on the walls of the vacutainer. Increased sample volume helps prevent toxicity that some low volume specimens experience due to high concentrations of heparin from the vacutainer. The second part of this system consists of two media for the *in vitro* culture of bone marrow cells—Bone Marrow Medium (Product Code B 6176) and Bone Marrow Medium Plus (Product Code B 6301).

Consistent culture media, optimal performance

During the development of Bone Marrow Medium, the effect of the basal medium formulation and all raw materials were examined to assess their impact on optimal cell growth and mitotic index. A variety of basal media formulations have been employed to culture bone marrow aspirates for cytogenetic analysis. We examined several different basal media, but were unable to determine significant differences in the performance of these media and elected to use RPMI-1640 as the base for the media reported here. Since undefined supplements used in media can exhibit significant lot-to-lot variability in terms of their contribution to medium performance, we established criteria for prescreening different lots of serum. Using a model system for evaluating serum, we confirmed the lot-to-lot variability of serum and were able to establish criteria for the screening and identification of material that provided optimal performance in the culture of specimens for cytogenetic analysis. The ability to prescreen materials employed in the manufacturing process provides the ability to control product consistency while ensuring optimal performance. The addition of conditioned medium to bone marrow aspirate cultures has been reported to improve the ability to culture specimens. Examination of the effects of the addition of Giant Cell Tumor (GCT) conditioned medium to the complete culture medium suggested a slight enhancement in growth but we were unable to determine the impact of the inclusion of GCT conditioned medium on cytogenetic accuracy. Although we were unable to verify their beneficial effects, the use of growth stimulants such as conditioned medium in bone marrow culture is still a source of considerable discussion. In recognition of this diversity of opinion, a second medium, Bone Marrow Medium Plus containing GCT conditioned medium, was also developed.

The final formulations were tested on a total of 50 human cases including normal and pathological bone marrow and leukemic blood specimens. Mitotic index was assessed using both qualitative estimation and metaphase counts. The quality of metaphases and improved chromosome morphology observed should facilitate case completion (Figures 1 and 2).

Improved transport medium

Based on our observations of the performance of the different media formulations examined it became apparent that only a limited degree of improvement in culturing bone marrow aspirates was possible through medium optimization. After reviewing the potential problems associated with bone marrow cultures, it was felt that the best opportunity for further improvement would be by improving the quality of specimens received by the testing laboratory. The variable size of specimens and the

relatively small specimen size in relation to the size of tubes used for transport can lead to several problems, including variation in the relative concentration of heparin in the sample and loss of specimen on the surface of the transport vessel. It was felt that placement of specimens in a physiologically supportive environment such as BMTS could serve potentially several functions. By controlling the level of heparin specimens are exposed to, as well as minimizing the loss of material on the transport container, use of a transport solution could decrease the impact of two shortcomings associated with specimen collection and transport. Product performance was assessed in studies using a total of 32 bone marrow specimens from human cases representing a wide variety of hematological disorders.

Figure 1. Normal metaphase spread using Bone Marrow Medium, Product Code B 6176.

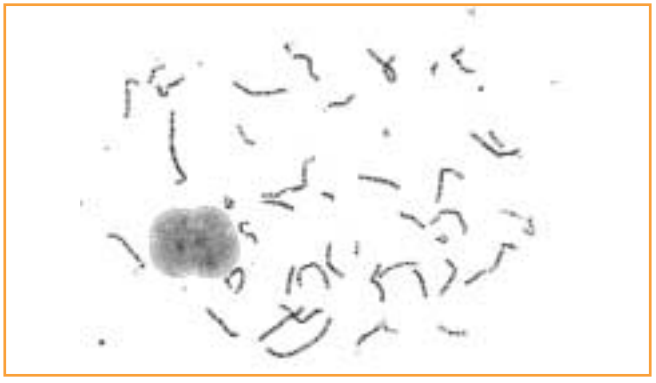

Figure 2. Abnormal metaphase spread using Bone Marrow Medium Plus, Product Code B 6301.

Specimens of 1 ml or less benefit the most from the use of BMTS. Additionally, the presence of sodium heparin in BMTS efficiently prevents clotting thereby minimizing specimen loss. Successful direct preparations can also be made from bone marrow specimens stored in BMTS for up to 72 hours. The growth characteristics of specimens stored in tubes with and without BMTS for up to five days were evaluated. Specimens stored in BMTS were able to be cultured more successfully and displayed better morphology compared to identical specimens shipped in identical tubes without BMTS. The quality and quantity of metaphases were evaluated by examining duplicate slides prepared from replicate cultures for mitotic index and quality of chromosome morphology. The quality of metaphases and improved chromosome morphology facilitates case completion (Figures 3 and 4).

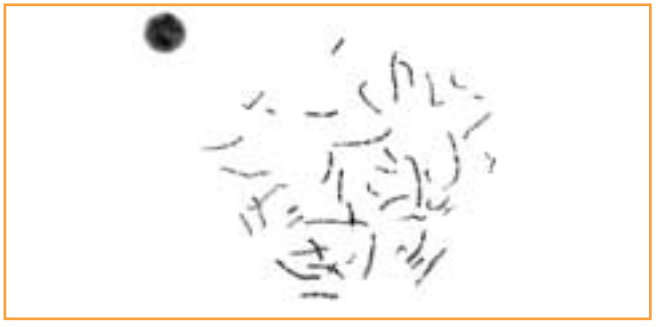

Figure 3. Normal metaphase spread stored in transfer solution for 48 hours.

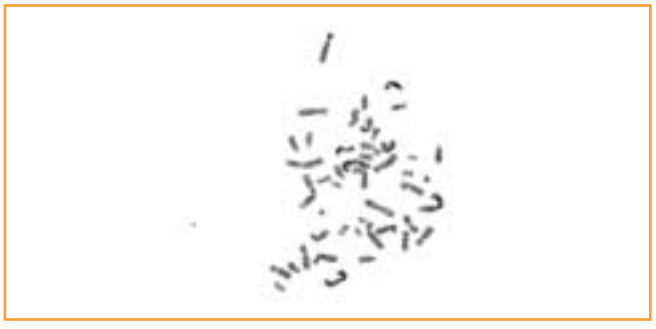

Figure 4. Normal metaphase spread stored without transfer solution for 48 hours.

Novel system superior to medium alone

Together these products represent a novel two-part system for the improved transport, recovery, and culture of bone marrow specimens for routine cytogenetic studies. Studies using a number of bone marrow specimens representing a wide variety of hematological disorders have shown that employing BMTS and culture medium in combination results in reduced failure rates, an increased mitotic index, as well as improved morphology, relative to the use of culture medium alone.

Ordering Information

Product	Description	Unit
B 6176	Bone Marrow Medium	100 ml 500 ml
B 6301	Bone Marrow Medium Plus	100 ml 500 ml
B 6426	Bone Marrow Transfer Solution	50 x 8 ml