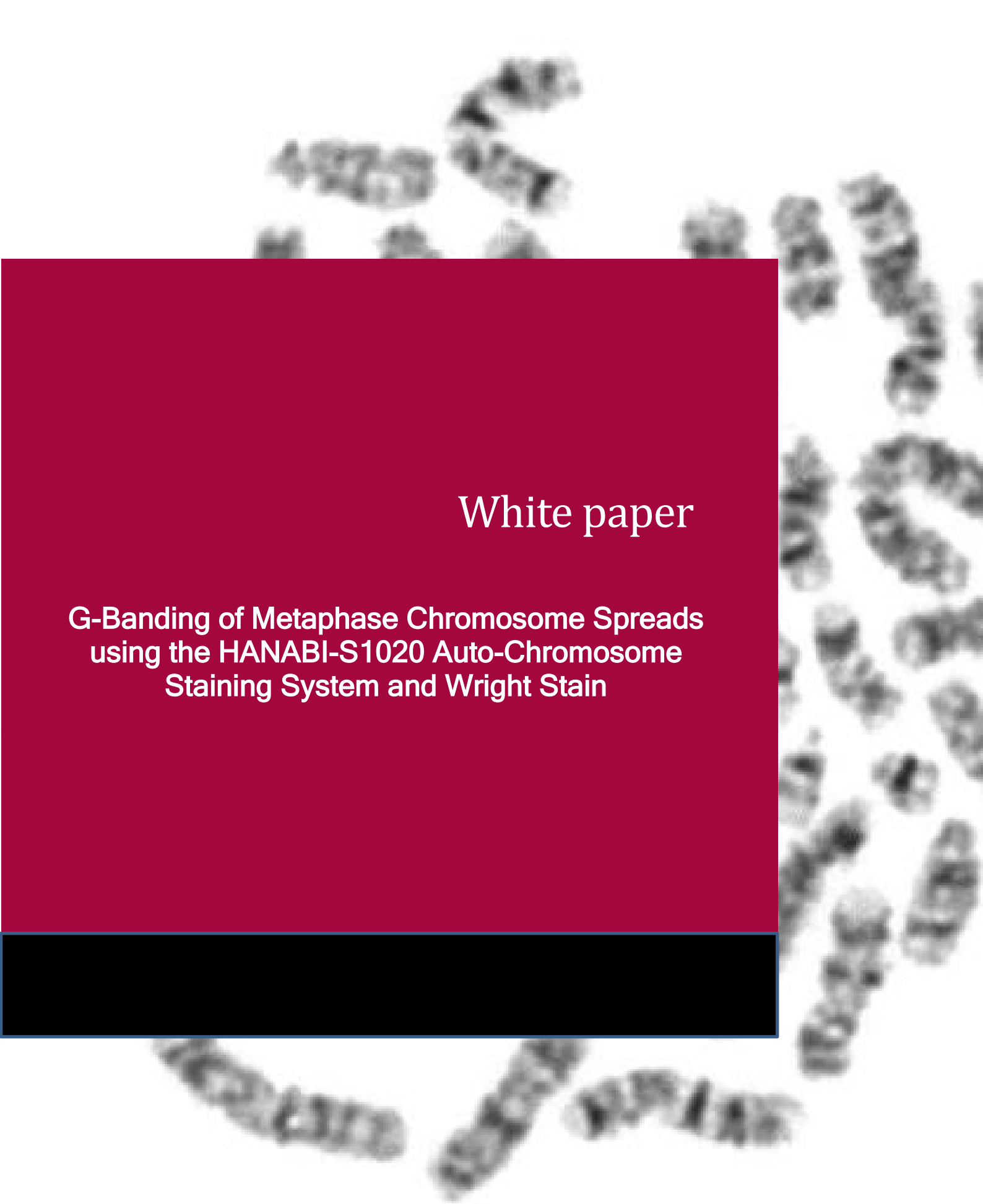

White paper

G-Banding of Metaphase Chromosome Spreads using the HANABI-S1020 Auto-Chromosome Staining System and Wright Stain

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G-Banding of Metaphase Chromosome Spreads using the HANABI-S1020 Auto-Chromosome Staining System and Wright Stain

Introduction

Efficient and consistent staining of metaphase chromosomes and interphase nuclei derived from peripheral blood or bone marrow samples plays a critical role in the day to day operation of clinical cytogenetic laboratories. Until now, the methods employed have largely relied on the manual skills of the cytogenetic technologist and the laboratory's ability to establish controlled and reproducible slide treatment methods employing a series of reagents for chemical treatment in successive steps.

A more advanced strategy involves automating the slide staining process using the HANABI-S1020 Staining System to deliver reproducible results of clinical diagnostic quality. The S1020 employs a patented UV-aging feature that dramatically reduces specimen processing time. For higher throughput labs, the UV-aging feature is critical, especially for labs that age for 12 - 24 hours. Manual staining protocols[1,2,3] depend on the time and skills of a dedicated and experienced cytogenetic technologist. When overwhelming numbers of samples need to be processed, automated staining provides a high-quality solution. Moreover, by automating the labs protocol(s), anyone in the laboratory can consistently initiate the process. ***Skilled technologists are then freed up to focus on analyzing metaphases.***

The present study was designed to:

(a) deliver reproducible G-banding results of diagnostic quality that are equivalent to standard manual protocols. The automated staining method must provide equivalent quality karyotyping images from blood and bone marrow specimens when compared with standard manual protocols as analyzed using a Leica based scanning system.

(b) determine what alterations in the staining protocol are required, if any, for the HANABI-S1020 to accommodate different spreading methods. Results were compared and evaluated based on overall quality.

HANABI-S1020 Auto-Chromsome Staining System and PVI Manual Metaphase Spreader

c) establish that the technology is mechanically sound and delivers uninterrupted performance. Determine if the HANABI-S1020 provides metaphase G-banding specimens consistently with high quality results.

(d) determine the time-savings achieved by using the HANABI-S1020 as compared to the lab's standard manual staining process, comparing the workflow time to get samples from the dropping stage to the microscope system with image capturing capabilities.

Background

The HANABI-S1020 Automated Staining System is designed to improve the quality, consistency and efficiency of the repetitive process of staining slide specimens. The system is capable of processing batches of 20 slides, and allows continual loading of additional slide racks, while the first batch is being processed. The Hanabi-S1020 incorporates an accelerated aging feature and accommodates most G-banding staining protocols using four reagent baths for the chemical and rinse treatments.

Materials & Methods

- HANABI-PII Metaphase Chromosome Harvester-
  Samples harvested using standard reagents & protocols
- HANABI-PVI Manual Chromosome Metaphase Spreader
- HANABI-S1020 Auto Chromosome Staining System

1. 0.3 % Wright Stain – Sigma PN 861375, weigh in slight excess 3 g Wright stain into 1 L anhydrous methanol (VWR Macron 3016-06). Prepared and filtered using standard protocols.
2. Wright Stain - dilute 50 mL 0.3 % Wright stain into 500 mL Gurr's buffer, pH 6.8.
3. 0.0025% Trypsin (no EDTA) – 2.5% trypsin, Gibco PN 15090046, thaw in fridge, aliquot into 2 mL tubes, store aliquots at -20 °C, thaw aliquot, dilute 0.5 mL in 499.5 mL 1X PBS.
4. 70% Ethanol – Sigma PN 45982, 350 mL + 150 mL DI water.

Specimen Summary

Specimen	Identification	Spreading	Staining
DSP-1	Control	Thermotron	Manual
DSP-2	Sample Split	Thermotron	HANABI-S1020
DSP-3	Sample Split	HANABI-PVI	HANABI-S1020
BME-1	Control	Thermotron	Manual
BME-2	Sample Split	Thermotron	HANABI-S1020
BME-3	Sample Split	HANABI-PVI	HANABI-S1020
ECT-1	Control	Thermotron	Manual
ECT-2	Sample Split	Thermotron	HANABI-S1020
ECT-3	Sample Split	HANABI-PVI	HANABI-S1020

Specimen Analysis Plan

(18) total metaphase images were collected using Leica Biosystems GSL-120 scanning microscope, (2) images from each specimen. Each Control slide was prepared using standard protocols using Wright stain. Sample splits were then spread using the Thermotron and HANABI-PVI Manual Spreader and stained using 0.03% Wright stain using the automated HANABI-S1020 Auto-Chromosome Staining System.

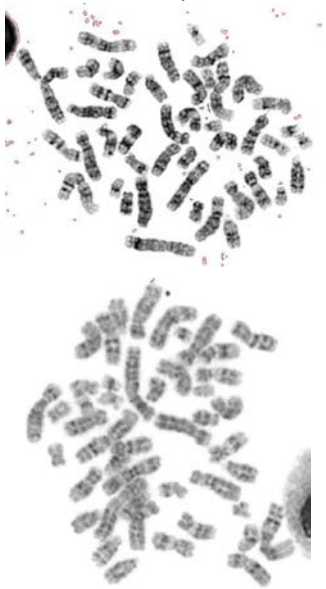
Standard Protocol

Results

Control Sample using Standard Protocol

Sample: DSP-1 met1\met2

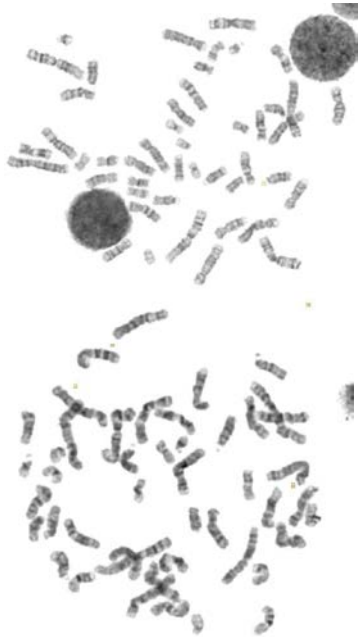
Type: Bone Marrow
Spreading: Thermotron 33-35%, RH 26 - 27 °C
Aging: Oven, 100 °C for 25 min.
Treatment: 6 sec. in 35 ml of 1X Trypsin-EDTA (Irvine Scientific Cat# 9341) diluted with 15 ml of 1X Hank's Balanced Salt Solution (HBSS, Irvine Scientific Cat #9220)
Staining: 1 part 0.3% Wright stain (in dry MeOH) diluted in 2 parts Gurr's buffer, stained for 50 sec., rinsed with tap water.



Result: Bone marrow specimen. Typical G-banded metaphases. Sharp, defined bands.

Sample: DSP-2-met1\met2

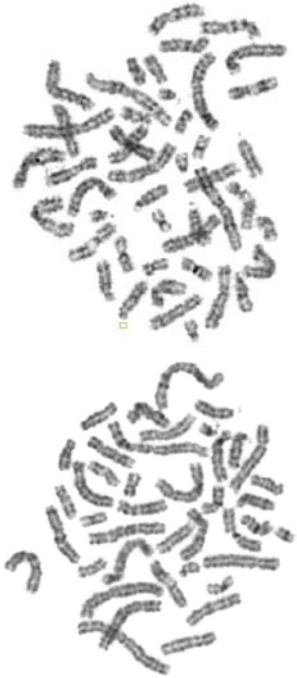
Type: Bone Marrow
Spreading: Thermotron 33-35% RH 26-27 °C
Aging - HANABI-S1020: 20 sec. UV exposure.
Treatment- HANABI-S1020: 4 min 0.0025% Trypsin (no EDTA) in 1X PBS
Staining: HANABI-S1020: 0.3% Wright stain (in dry MeOH) diluted 1:10 in Gurr's buffer (pH 6.8), stained for 3 min.



Result: Comparable chromosome spreading and banding.

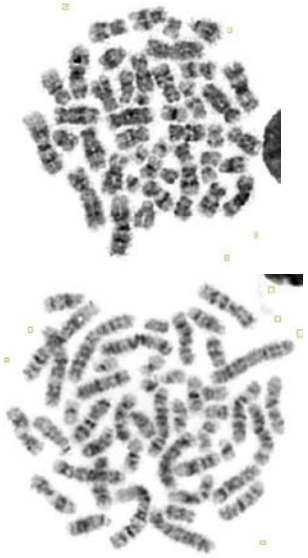
Sample: DSP-3-met1\met2

Type: Bone Marrow
Spreading: HANABI-PVI: drop 30 uL, 120 sec dry, 10-deg. tilt Dry Index = 9.6(%RH 54, T = 31 °C)
Aging - HANABI-S1020: 20 sec. UV exposure.
Treatment: HANABI-S1020: 4 min 0.0025% Trypsin (no EDTA) in 1X PBS
Staining: HANABI-S1020: 0.3% Wright stain (in dry MeOH) diluted 1:10 in Gurr's buffer (pH 6.8), stained for 3 min.



Result: Sharp, defined bands.

Control Sample using Standard Protocol
Sample: BME-1-CONTROL-met1\met2
Type: Bone Marrow
Spreading: Thermotron 33-35%, RH 26 - 27 °C
Aging: Oven, 100 °C for 25 min.
Treatment: 6 sec. 35 ml of 1X Trypsin-EDTA (Irvine Scientific Cat# 9341) diluted with 15 ml of 1X Hank's Balanced Salt Solution (HBSS, Irvine Scientific Cat #9220))
Staining: 1 part 0.3% Wright stain (in dry MeOH) diluted in 2 parts Gurr's buffer, stained for 50 sec., rinsed with tap water.

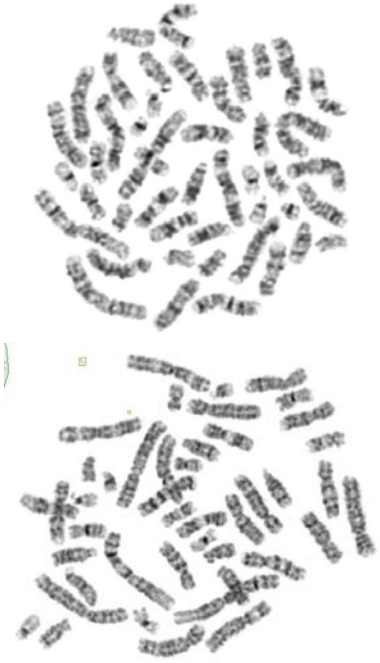


Result: Adequate banding, chromosomes slightly mushy.

Sample: BME-2-met1\met2
Type: Bone Marrow
Spreading: Thermotron 33-35% RH 26-27 °C
Aging - HANABI-S1020: 20 sec. UV exposure.
Treatment- HANABI-S1020: 4 min 0.0025% Trypsin (no EDTA) in 1X PBS
Staining – HANABI-S1020: 0.3% Wright stain (in dry MeOH) diluted 1:10 in Gurr's buffer (pH 6.8), stained for 3 min.

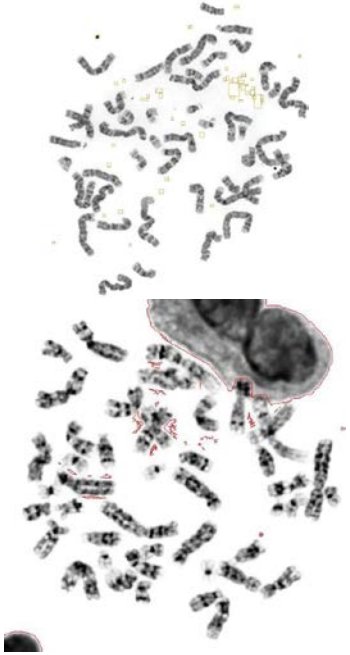
Result: Sharp, defined bands.

Sample: BME-3-met1\met2
Type: Bone Marrow
Spreading: HANABI-PVI, drop 30 uL. 120 sec dry, 10-degree tilt, Dry Index 9.6 (%RH 54, T = 31 °C)
Aging - HANABI-S1020: 20 sec. UV exposure.
Treatment- HANABI-S1020: 4 min 0.0025% Trypsin (no EDTA) in 1X PBS
Staining – HANABI-S1020: 0.3% Wright stain (in dry MeOH) diluted 1:10 in Gurr's buffer (pH 6.8), stained for 3 min.



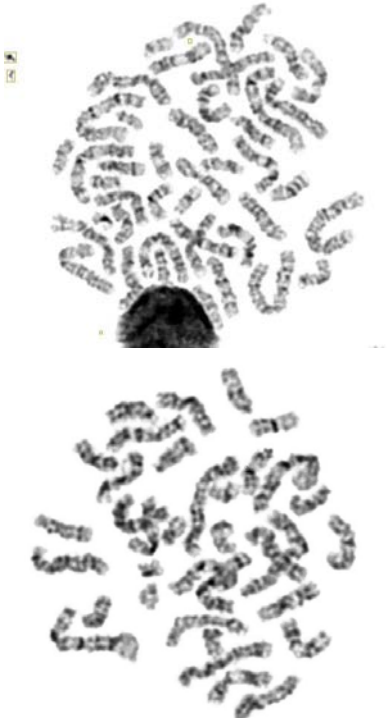
Result: Sharp, defined bands.

Control Sample using Standard Protocol
Sample: ECT-1-CONTROL-met1\met2
Type: Bone Marrow
Spreading: Thermotron 33-35%, RH 26 – 27 °C
Aging: Oven, 100 ° C for 25 min.
Treatment: 6 sec. 35 ml of 1X Trypsin-EDTA (Irvine Scientific Cat# 9341) diluted with 15 ml of 1X Hank's Balanced Salt Solution (HBSS, Irvine Scientific Cat #9220))
Staining: 1 part 0.3% Wright stain (in dry MeOH) diluted in 2 parts Gurr's buffer, stained for 50 sec., rinsed with tap water.



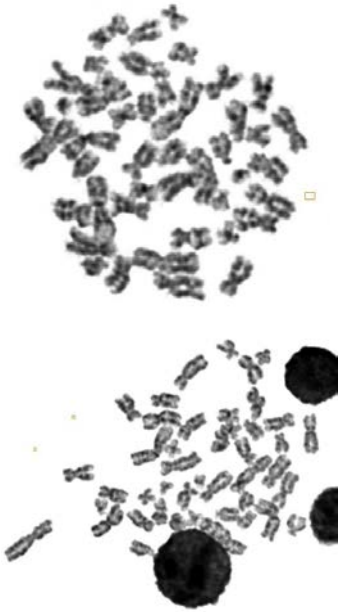
Result: Typical results with manual standard protocol.

Sample: ECT-2-met1\met2
Type: Bone Marrow
Spreading: Thermotron 33-35%, RH 26 – 27 °C
Aging - HANABI-S1020: 20 sec. UV exposure.
Treatment- HANABI-S1020: 4 min 0.0025% Trypsin (no EDTA) in 1X PBS
Staining – HANABI-S1020: 0.3% Wright stain (in dry MeOH) diluted 1:10 in Gurr's buffer (pH 6.8), stained for 3 min.



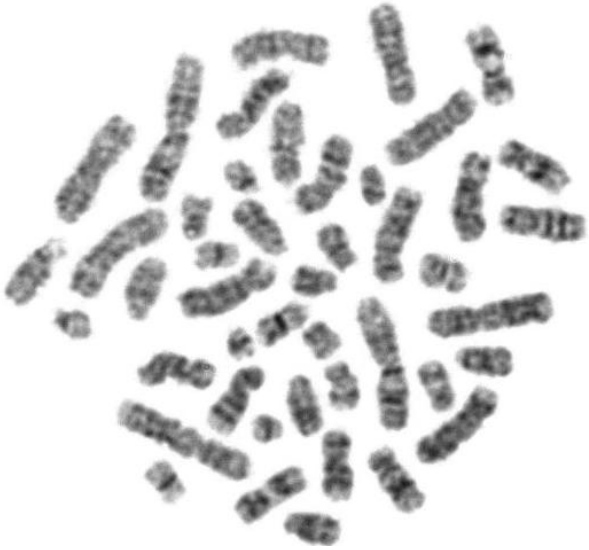
Result: Comparable chromosome spreading and banding.

Sample: ECT-3-met1\met2
Type: Bone Marrow
Spreading: HANABI-PVI, drop 30 uL. 120 sec dry, 10-deg. tilt, Dry Index 9.6 (%RH 54, T = 31 °C)
Aging - HANABI-S1020: 20 sec. UV exposure.
Treatment- HANABI-S1020: 4 min 0.0025% Trypsin (no EDTA) in 1X PBS
Staining – HANABI-S1020: 0.3% Wright stain (in dry MeOH) diluted 1:10 in Gurr's buffer (pH 6.8), stained for 3 min.



Result: Borders on chromosomes are undefined and mushy, attributable to harvested sample.

Standard Protocol vs HANABI-S1020 Automated Staining

BME-1-CONTROL-met2 Spreading \ Staining Thermotron \ Manual Staining	BME-2-met1 Spreading \ Staining Thermotron \ HANABI-S1020	BME-3-met1 Spreading \ Staining HANABI-PVI \ HANABI-S1020
		

HANABI-S1020 G-Banding Summary

I) Deliver reproducible G-banding results of diagnostic quality that are equivalent to standard protocols. The automated staining method must provide equivalent quality images that are suitable for karyotypic analysis in blood and bone marrow specimens.
Conclusion: *Operational settings were determined to achieve comparable quality.*

II) Determine what alterations in the staining protocol were required for the HANABI-S1020 to accommodate different spreading methods. Results were compared and evaluated based on overall quality.
Conclusion: *A single HANABI-S1020 protocol achieved diagnostic quality with diluted Wright Stain for both peripheral blood and bone marrow sample types regardless of the spreading methods employed.*

III) Establish that the Hanabi-S1020 technology is mechanically sound and delivers uninterrupted performance. Determine if the HANABI-S1020 achieves consistent, high-quality staining of metaphases.
Conclusion: *Quality results were obtained using identical operational settings on the Hanabi-S1020 regardless of spreading method (slides dropped with Hanabi-PVI comparable to those prepared within Thermotron Chamber).*

IV) Determine the time-saving value of the HANABI-S1020 when compared to the lab's standard protocols providing an estimate of the time to get samples from dropping stage to the auto-scanning microscope system.
Conclusion: *Time savings can be achieved for most protocols and varies with the number of samples to be processed. A single protocol can be used with the HANABI-S1020 compared to protocols that vary based on the type of sample processed for the standard protocols. See Appendix I*

Standard Protocol used for Reference Analysis:

Spreading: Thermotron 33 - 35%, RH 26 – 27 °C
Aging: Oven, 100 ° C for 25 min.
Treatment: 6 sec. 35 ml of 1X Trypsin-EDTA (Irvine Scientific Cat# 9341) diluted with 15 ml of 1X Hank's Balanced Salt Solution (HBSS, Irvine Scientific Cat #9220))
Staining: 1 part 0.3% Wright stain (in dry MeOH) diluted in 2 parts Gurr's buffer, stained for 50 sec. rinsed with deionized water.

Appendix I

Table 1: Comparison of Total Sample Processing Time for Staining.

Method Time, min	1 Slide	5 Slides	10 Slides	15 Slides	20 Slides
Manual Blood culture	41.8	42.2	44.2	46.3	48.0
Manual Oncology	26.1	26.3	27.5	28.7	29.7
Hanabi-S1020	**15.4**	**15.4**	**19.7**	**24.0**	**28.4**

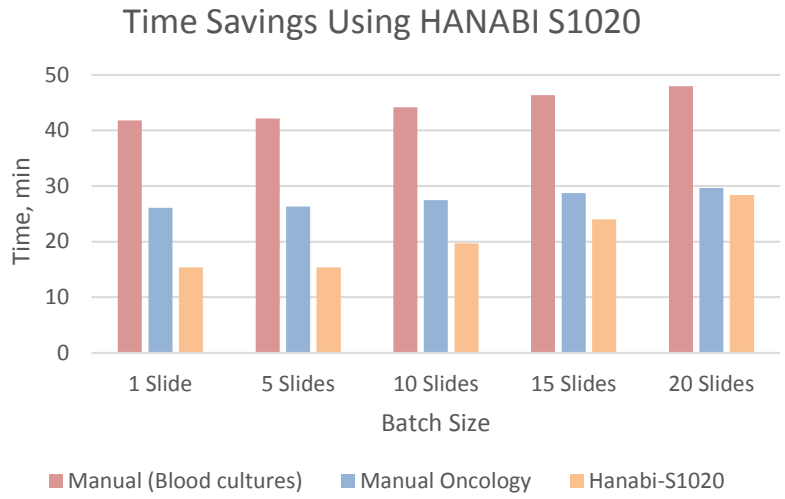


Figure 1: Time savings comparison of HANABI-S1020 with manual standard protocol.

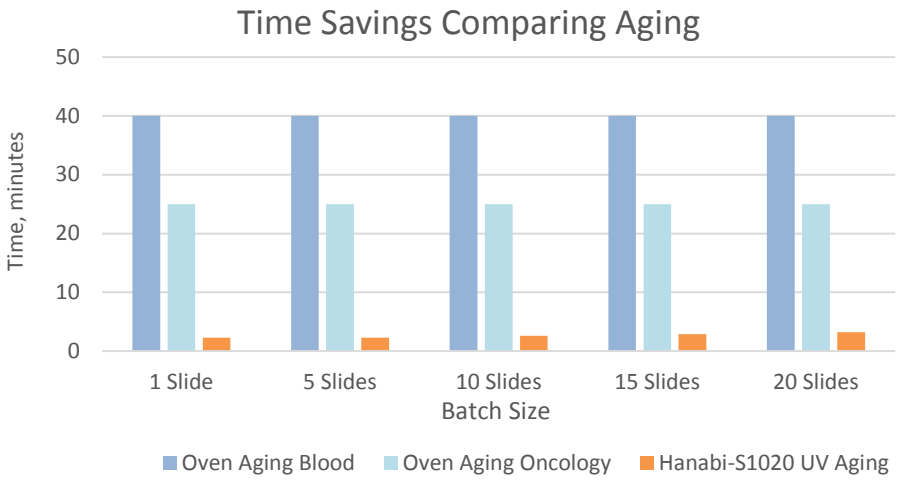


Figure 2: Time savings of aging with UV compared to using an oven.

Table 2: Summary of detailed time tables; Manual Protocol and HANABI-S1020.

Timing - Detailed, min					
Manual (Blood cultures)	**1 Slide**	**5 Slides**	**10 Slides**	**15 Slides**	**20 Slides**
Oven aging	40.0	40.0	40.0	40.0	40.0
Reagent Preparation	2.0	5.0	5.0	5.0	5.0
Trypsinize	0.2	0.5	0.8	1.5	1.7
Stain	1.5	1.5	3.0	4.5	6.0
Water rinse + dry	0.2	0.2	0.3	0.3	0.3
Multitask offset*	-2.0	-5.0	-5.0	-5.0	-5.0
Total Time, min	41.8	42.2	44.2	46.3	48.0

Manual (Oncology)	**1 Slide**	**5 Slides**	**10 Slides**	**15 Slides**	**20 Slides**
Oven aging	25.0	25.0	25.0	25.0	25.0
Reagent Preparation	2.0	5.0	5.0	5.0	5.0
Trypsinize	0.1	0.3	0.5	0.9	1.0
Stain	0.8	0.8	1.7	2.5	3.3
Water rinse + dry	0.2	0.2	0.3	0.3	0.3
Multitask offset*	-2.0	-5.0	-5.0	-5.0	-5.0
Total Time, min	**26.1**	**26.3**	**27.5**	**28.7**	**29.7**

Hanabi-S1020	**1 Slide**	**5 Slides**	**10 Slides**	**15 Slides**	**20 Slides**
Program initiate	0.5	0.5	0.5	0.5	0.5
Reagent Preparation	5	5	5	5	5
UV stabilize	2	2	2	2	2
UV aging	0.3	0.3	0.6	0.9	1.2
Trypsin dip	4.1	4.1	4.1	4.1	4.1
Ethanol rinse	0.2	0.2	0.2	0.2	0.2
Stain	3.1	3.1	3.1	3.1	3.1
Water rinse + dry	0.2	0.2	0.2	0.2	0.2
Stainer time offsets**	0	0	4	8	12.1
Total Time, min	**15.4**	**15.4**	**19.7**	**24**	**28.4**

**Multitask offset: Preparation of staining reagents is conducted during the Oven Aging, so this time is not additive to overall processing time. Conversely, this time is additive for the HANABI-S1020. If timely, this preparation could be completed during the final stage of Harvest prior to spreading.*

***Staining System time offsets: within the HANABI-S1020, multiple slide racks are processed in parallel baths to achieve faster throughput, so bath times are not linearly additive for total throughput time.*

Table 3: HANABI-S1020 Routine operation table and timing.

HANABI-S1020 with HANABI-PVI Spreading –
Total Time Required to Setup, Spread and Stain

Method Time, min	**1 Slide**	**5 Slides**	**10 Slides**	**15 Slides**	**20 Slides**
Reagent Prep\S1020 Setup	17.00	17.00	17.00	17.00	17.00
Spread using HANABI-PVI	5.00	12.00	20.00	25.00	35.00
S1020 Aging\Staining	**15.40**	**15.40**	**19.70**	**24.00**	**28.40**
Total Time	**37.40**	**44.40**	**56.70**	**66.00**	**80.40**

References:

1. Sanchez, O., I.I. Escobar, J.J. Yunis. A Simple G-banding Technique, The Lancet, pp. 269, August 4, 1973.
2. Spurbeck, J.L., Zinsmeister, A.R., Meyer, K.J., Jalal, S.M., The Dynamics of Chromosome Spreading, American Journal of Medical Genetics, 61:387-393, 1996.
3. Spurbeck, J.L., Carson, R.O., Allen, J.E., Dewald, G.W., Culturing and Robotic Harvesting of Bone Marrows, Lymph Nodes, Peripheral Blood, Fibroblast and Solid Tumors with in situ Techniques, Cancer Genetics and Cytogenetics, 32: 59-66, 1988.