Performance and Cost Benefit Analysis for Fluorescence *in Situ* Hybridization Automation for Clinical Cytogenetics

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Abstract

In clinical cytogenetics, fluorescence *in situ* hybridization (FISH) assay is routinely used for detection of various genetic abnormalities. It is a robust and reliable technique to diagnose, characterize, and stage hematological malignancies. This study assessed the advantages of pre-and post-treatment automation of FISH slide preparations using the Abbott VP 2000 Processor. Slides were prepared using manual and automated methods. They were analyzed and compared to one another for the overall quality and concordance of the scoring results. Additionally, we compared the total protocol run time for both methods, performed a cost analysis considering the technologist's "hands-on" time, and the amount of reagents used per week. We found a significant labor cost savings for a VP 2000 Processor protocol, and shorter time for both pre-and post-treatment steps compared to a manual method. However, reagents cost per week was lower for a manual method. The quality of slides for both methods did not significantly differ from one another, and scoring results were in concordance. This study reveals that a VP 2000 Processor is a cost and time effective instrument, suitable for a high throughput laboratory.

Introduction

- Fluorescence *in situ* hybridization (FISH) is a powerful tool for identification of chromosomal aberrations in clinical cancer cytogenetics.
- For hematological studies, white blood cells are fixed on a glass slide and pre- treated (aged and dehydrated) with various reagents. Fluorescently labeled probes (short pieces of DNA) are applied on a pre- treated slide, cover- slipped and hybridized under a specific conditions.
- For visualization under a microscope, slides must be washed to remove excess probe and non-specific hybridization, counterstained with the DAPI to visualize the nucleus.
- The assay is robust but required pre-and post-treatments of the slides are repetitive and time-consuming tasks.
- A VP 2000 Processor is an automated instrument designed for pre- and post- treatment of FISH slides.

Goal of the Study

- Evaluate the performance of the VP 2000 Processor
- Assess quality of the slides by comparing with the manual method
- Calculate labor and reagent cost
- Develop optimal operational protocol

Methods

- Two sets of slides were prepared to hybridize FISH probes for the following cancer disorders: multiple myeloma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, myelodysplastic syndrome, and lymphoma.
- One set was processed using manual protocol, another using VP 2000 protocol.
- Slides were examined microscopically and compared for signal intensity (diminished, moderate, or bright), background (present or not), and concordance of scoring results (normal or abnormal).
- Kendall's *W* coefficient of concordance, Cohen's *k* kappa coefficient, and *p*- values were calculated using IBM SPSS Statistics software.
- Reagent cost necessary per week was calculated and compared to that of the manual method.
- Manual labor cost and time analysis for both methods were compared.
- The time duration of protocols for both methods were compared.

Results

- **Table 1.** Summary of Kendall's *W* coefficient of concordance, Cohen's kappa coefficient *k,* and *p*- values of various FISH cancer panels

Cancer Panel	# cases	# probes	Cohen's kappa *k, p*- values: signal intensity	background	scoring results	Kendall's *W, p*- values
MM	5	7	*k* = .76, *p* < .001	*k* = .40, *p* = .019	*k* = 1, *p* < .001	*W* =.84, *p* <.001
MDS	5	4	*k* = .89, *p* < .001	*k* = .22, *p* = .334	*k* = 1, *p* < .001	*W* =.93, *p* <.001
ALL	3	6	*k* = .31, *p* = .180	*k* = -.06, *p* = .803	*k* = 1, *p* < .001	*W* =.90, *p* <.001
CLL	5	6	*k* = .73, *p* < .001	*k* = .52, *p* = .004	*k* = 1, *p* < .001	*W* =.90, *p* <.001
LYMPHOMA	5	2	*k* = 1, *p* < .001	*k* = 1, *p* < .001	*k* = 1, *p* < .001	*W* =.90, *p* <.001

Note. MM- multiple myeloma, MDS- myelodysplastic syndrome, ALL- acute lymphoblastic leukemia, CLL- chronic lymphocytic leukemia. # cases- total cases, # probes- number of probes in the FISH panel

Graphical representation of cost and time analysis

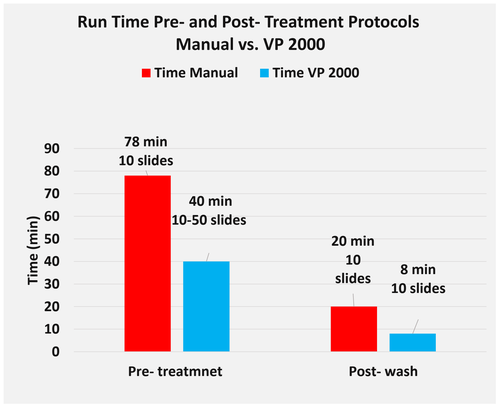


Figure 1. Duration of the protocols to process one batch of slides in one run.

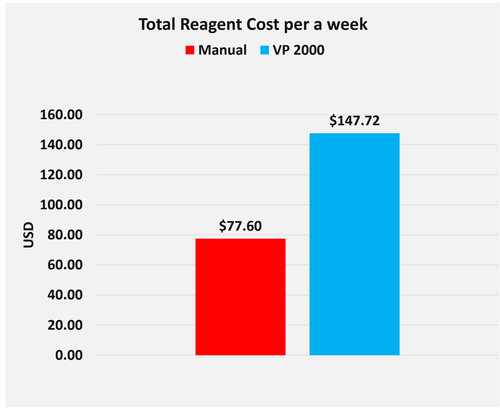


Figure 2. Cost of reagents calculated based on the volumes necessary to run the protocols per one week.

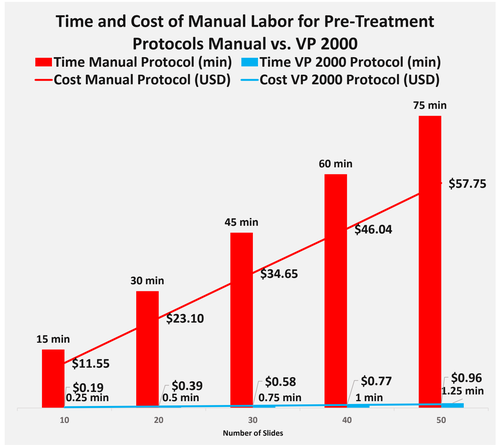


Figure 3. Cost of manual labor calculated based on the mean hourly rate of cytogenetic technologist multiplied by the approximated time necessary to process one batch of 10 to 50 slides.

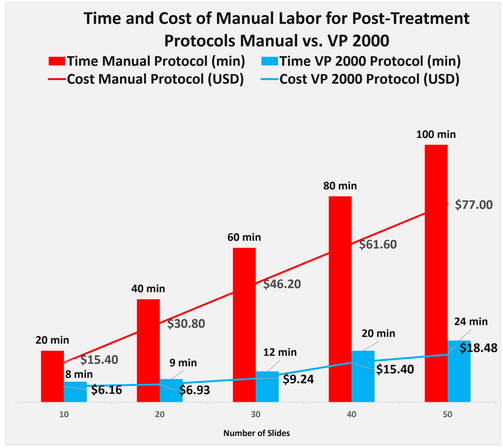


Figure 4. Cost of manual labor calculated based on the mean hourly rate of cytogenetic technologist multiplied by the approximated time necessary to process batch of 10 to 50 slides.

Acknowledgements

I am deeply grateful to my colleagues and Dr Gloria Viboud for their assistance with this project

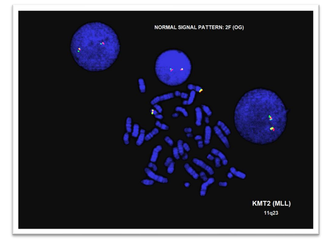
Conclusions

- The results of our study demonstrate the significant time and cost savings of automation of FISH slide processing for a high throughput cytogenetic laboratory
  - The combined cost of reagents and manual labor per a batch of 50 slides for the automated method was $167.16, whereas for the manual method was $212.35
  - The total "hands-on" time to process 50 slides was 26.25 min for the automated and 175 min for the manual
  - Shorter protocol run time for automated compared to that of manual, 40 min (10-50 slides) to 78 min (10 slides)
- A VP 2000 Processor produced a high-quality slides, comparable to the slides of the manual protocol.

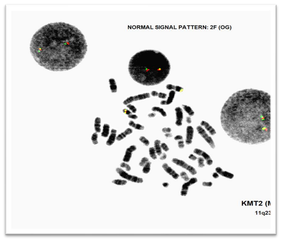
Discussion

- The cost of reagents required for a VP 2000 protocol was greater than that for manual method due to size of the basins of the instrument: 150 mL, 250 mL and 500 mL used to process the batch of 8, 20 and 50 slides, respectively. For the manual protocol 50 mL Coplin jars that fit 10 slides were used.
- Hands-on time was significantly less for a VP 2000 Processor, as it is a truly walk-away instrument. The technologist manipulates the slides only once, when loading the slide holder.
- Calculations of Kendall's *W* coefficient of concordance indicate strong agreement between the manual and automated methods.
- Calculations of Cohen's kappa *k* for the background noise showed slight to moderate agreement due to subjective nature of the task. Cohen's kappa for signal intensity was at moderate to near perfect, and scoring results were at perfect agreement.
- The total run time was shorter for a VP 2000 Processor, since the new modified and shortened protocol, based on the manual method, was established empirically prior to the study.

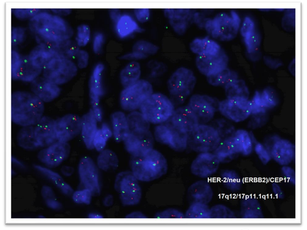
Examples of FISH Images



A



B



C

Figure 7. *A* and *B.* DAPI- stained and inverted interphase and metaphase human bone marrow cells. Locus specific dual color break apart probe labeled with orange and green. Chromosome 11 (*MLL* gene segment on a long arm at q23 band). *C* . Amplified HER-2/neu (labeled with an orange) paraffin embedded breast tissue. Centromere of chromosome 17 is labeled with a green color