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**OF PROJECT: ASSESSMENT OF NEW STRATEGIES FOR SCREENING OF B**  
**FOR HCV**

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## PROJECT

# **ASSESSMENT OF NEW STRATEGIES FOR SCREENING OF BLOOD FOR HCV**

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## **I. TECHNICAL PROGRESS**

### **I. A) Research Objectives**

The main objective of the study is to improve accuracy of screening blood donations for HCV.

The objectives for the first six months:

1. To train lab technicians to work with the HCV-Ag kit.
2. To obtain all the necessary equipment and supplies needed for the study experiments in the study sites.
3. To plan the different stages of the project, and create proper protocols for each stage for each laboratory.
4. To perform the preliminary experiments.
5. To perform the proficiency panel.

### **I. B) Research Accomplishments**

During the reported period , we have accomplished the following items of the work plan:

- (1) Trained lab technicians to handle the HCV-Ag kit (see section II.C) .
- (2) Obtained the necessary equipment required, and initiated contact with the suppliers of kits, seroconversion panels and HCV positive blood samples. So far we have purchased an HCV-Ag assay kit and 7 HCV seroconversion panels.
- (3) Planned the different stages of the study (as specified below).
- (4) Developed protocols for the preliminary experiments, the proficiency panel and the seroconversion panels (as specified on pages 6-11).

*below is a brief description of the activities in each study site:*

### Outline of the study plan:

This study will be done in the following stages:

1. Preliminary experiment on 15 HCV-Ag positive samples.
2. Proficiency panel.
3. Seroconversion panels.
4. Testing pools from negative donors with implanted positive samples.

### Detailed description and purpose of each stage:

**1. The preliminary experiments** will include pooling with 15 HCV-Ag known positive samples. These samples will be pooled in pool sizes of 12, 6 and 3 and tested for HCV with the HCV-Ag assay. These experiments will provide us with a first indication on whether the HCV-Ag assay is capable of detecting positive signals in pools, and the maximal pool size that can be used in the forthcoming experiments. This stage will be done at MDA, and the pooling will be done manually.

The protocol of the preliminary experiments is described in pages 6-7.

If these experiments will demonstrate that the HCV-Ag kit is not capable of detecting positive signals in the pools, we will have to reconsider the objectives of the study.

We have already started working on these experiments, and the summary of the results (which seem to be promising) will be presented in the next report.

**2. Proficiency panel** – This stage will serve as a quality control, ensuring that all study sites perform the assay and the pooling with similar proficiency. The panel will be prepared at BGU, and will be pooled manually and tested at BGU, MDA and EPRI. We will conduct this stage after all the lab technicians in all the study sites are trained and capable of working with the kit.

The protocol of the proficiency panel is described on page 8.

**3. The experiments on seroconversion panels** – In this stage, details of PCR results and

At this stage the pooling will be done manually.

Pools containing a seroconversion panel bleeding which is PCR positive in singleton will be transferred to MDA and tested for HCV-RNA by PCR.

The protocol of the seroconversion panels stage is described in pages 9-11.

**4. Testing pools from negative donors from routine work** – The negative donor samples will be pooled with implanted positive samples, and tested with the HCV-Ag assay including the confirmatory tests. This stage will be pooled with the automated sampler when done at MDA, and pooled manually when done at EPRI. Positive samples and pools containing positive samples will be transferred to MDA for HCV-RNA testing.

The protocol of the preliminary experiments:

**Preliminary experiments of pooled ELISA testing for HCV-Ag with pool sizes of 12-6-3: Standards of procedures for work at MDA**

**Goal:**

To get a preliminary indication on which pool sizes are possible for detection by the HCV-Ag assay (Ortho company). After reviewing the results of this stage, the researchers will decide whether to proceed with the existing objectives and protocols of the study.

**Materials:**

- 15 samples that are HCV-Ag positive and HCV-Ab negative. These samples are left over from experiments done at MDA 2 years ago, and have been kept frozen at  $-30^{\circ}\text{C}$ . Five of these samples are considered "strong" positives, 5 are considered "intermediate" and 5 are considered as "weak" positives, according to the HCV-Ag result they had in singleton testing.

The "strong" positives gave an OD result that was above 1.5.

The "intermediate" positives gave an OD result that was between 0.5 and 1.5.

The "weak" positives gave an OD result that was between the cutoff point and 0.5.

- 66 samples negative for anti-HCV and HCV-Ag, obtained from the routine work of MDA during May-July 2003.

**Methods:**

**Pooling:**

1. The first 5 positive samples will be pooled with 22 HCV-Ag negative samples in pool sizes of 12, 6 and 3. For this stage, we will take the positive samples which are considered "strong" positives. 3 positive samples will be pooled with a set of 11 negatives, and the other 2 positive samples will be pooled with a different set of 11 negatives. Assigning the positive samples to the negatives which will pool them, will be done at random.
2. The next 10 positive samples will be pooled. Pool sizes will be determined after examining

The pooling process:

Negative samples will be taken from routine work at MDA. The samples will be negative for HCV-Ab , and will be tested for HCV-Ag in singleton to assure that they are HCV-Ag negative. Each pool of 12 will include sera from the 11 negative samples and from one positive sample. If a sufficient amount of serum remains from the positive sample, we will make pools of 6. Each pool of 6 will include sera from 5 of the negative samples and from one positive sample. If there is still enough serum from the positive sample, we will also make pools of 3. Each pool of 3 will include sera from 2 negative samples and from one positive sample. The volume of each singleton sample and pool size will be 100 $\mu$ l, which is the volume required for the HCV-Ag assay.

Testing:

HCV-Ag assay testing will be performed in each pool size formed.

Results' documentation:

The results of the singleton HCV-Ag assay and PCR results for the HCV-Ag positive samples will be obtained from the previous testing performed at MDA.

The results of the preliminary experiments will be presented in the following table:

**Preliminary experiments with HCV-Ag: HCV-Ag (ORTHO) pooled testing compared to HCV-Ag (ORTHO) and PCR in singleton**

	sample	PCR in singleton	HCV-Ag S/CO in singleton	HCV-Ag, S/CO		
				in pools of 3	in pools of 6	in pools of 12
<b>strong</b>	<b>1</b>					
	<b>2</b>					
	<b>3</b>					
	<b>4</b>					
	<b>5</b>					
<b>intermediate</b>	<b>6</b>					
	<b>7</b>					

The protocol of the proficiency experiments:

**Proficiency panels: Standards of procedures**

**Goal:**

To demonstrate that all three study site laboratories (MDA, BGU, and EPRI) perform the HCV-Ag assays and manual pooling with similar proficiency.

**Materials:**

- Three samples that are HCV-Ag positive and HCV-Ab negative, taken from a seroconversion panel.
- 69 samples negative to anti-HCV and HCV-Ag, obtained from the routine work of MDA.

**Panel Description:**

Each of the 72 Eppendorf tubes should have a volume of 400µl serum in each, numbered 1-72.

The panel will be arranged in such a way that 3 pools of 12 should be positive and 3 pools of 12 should be negative.

Note: These panels will be assembled by Dr. Arie Yaari. All persons testing the proficiency panel will be blinded.

**Shipping and storage conditions**

The panels should be kept in  $-70^{\circ}\text{C}$  and shipped on dry ice to each of the other two study sites.

**Testing the Proficiency Panel**

- 1) Pool 4 pools of 12 in order. Test once.
- 2) Pool 8 pools of 6. Test once.
- 3) Pool 16 pools of 3. Test once.
- 4) Test individual samples belonging to the positive pools of 3.
- 5) Report all results.

***Directions for manual pooling in pool sizes of 12, 6, 3***

The protocol of the seroconversion panels stage:

**Seroconversion panels: Standards of procedures**

**Goal:**

- 1) To measure the sensitivity and specificity of pooling procedure for HCV-Ag for pool sizes 3, 6 and 12 by HCV-Ag assay (Ortho company) compared to singleton testing for HCV-Ag.
- 2) To measure the sensitivity and specificity of pooling procedure for HCV-Ag for pool sizes 3, 6 and 12 by HCV-Ag assay (Ortho company) compared to pooled testing by PCR.

**Materials:**

- seroconversion panels for HCV (purchased from Impath-Bio Clinical Partners, BBI and Nabi Diagnostics companies)
- Anti-HCV and HCV-Ag negative samples (obtained from the routine screening of blood donations at MDA).

**Methods:**

Pooling:

All bleedings from each panel will be pooled in pools of sizes 3, 6 and 12 anti-HCV and HCV-Ag negative samples.

For each dilution of a panel bleeding, we will need 11 negative samples chosen randomly from the range of negative samples available from the MDA Blood services routine screening procedures.

The dilution process:

Label all 12 samples by numbers 1-12, in a way that the bleeding from the panel will be #1.

For pooling in pool size of 12, use all 12 samples and create 2 identical pools in 2 independent test tubes (duplicate).

For pooling in pool size of 6, use samples 1-6 for the first 2 identical pools, and

- (1) The 11 samples which will be used for pooling each panel should be HCV-Ag negative, so they will be tested for HCV-Ag to assure this.
- (2) Tests for HCV-Ag will be performed in singleton for panel bleedings.
- (3) The pools will be tested for HCV-Ag.
- (4) HCV-Ag confirmatory test will be done on every singleton and pool, which has a positive result in the HCV-Ag assay.
- (5) PCR testing will be performed for all pools that contain the panel bleedings, which were PCR positive in singleton (according to the panel's catalog).

Results' documentation: We will document all the results of testing, as well as the serological status of the samples before they enter the experiments (according to MDA blood services testing), using MDA's computerized data base.

Laboratory volume calculations:

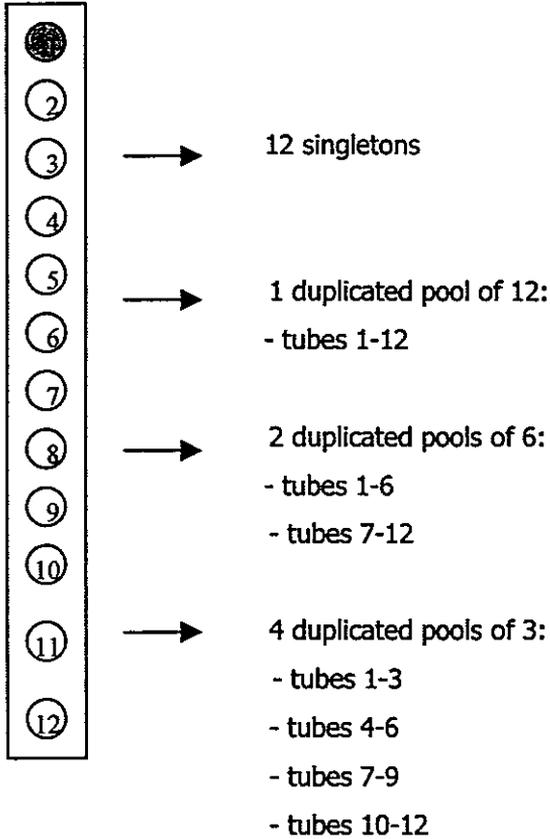
Each singleton sample and pool will need to have the total volume of 900ul, calculated as follows:

- |                                                                             |       |
|-----------------------------------------------------------------------------|-------|
| (1) HCV-Ag assay -----100μl (possibly done 3 times, if found positive)----- | 300μl |
| (2) HCV-Ag confirmatory test -----*2-----                                   | 200μl |
| (3) PCR testing -----                                                       | 200μl |
| (4) Archive -----                                                           | 200μl |

Each panel bleeding will be used for singleton testing and pooling. Each panel bleeding will need to have the total volume of 1950ul, calculated as follows:

- |                                                                   |       |
|-------------------------------------------------------------------|-------|
| (1) for singleton testing ----- 900 -----                         | 900μl |
| (2) for testing in pools of 12 ----- 900/12 ----- 75μl --*2 ----- | 150μl |
| (3) for testing in pools of 6 ----- 900/6 ----- 150μl --*2-----   | 300μl |

One panel bleeding and 11 negative samples



### **I. C) Scientific Impact of Cooperation**

The cooperative activities between the different study sites at this stage are realized in the different planning sessions (see appendix 1) and training sessions (as described in section II.C).

### **I. D) Description of Project Impact**

It is too early to report on the project impact as we are at the very initial stages of the study. However, we do expect that the results obtained from this project will make changes in the routine work of blood banks of this region, and improve blood screening.

### **I. E) Strengthening of Middle Eastern Institutions**

Training investments:

Currently, there is no use of HCV-Ag kits in the routine work at any of the study sites. The knowledge in using this kit is important and helpful to all three collaborating institutions in the region.

### **I. F) Future Work**

We will complete the training of all lab technicians. We expect that this task will be easier now that the political situation between Israel and the Palestinian Authority has improved.

The experiments for the next six months will be performed according to the proposed protocols (see detailed protocols on pages 6-11) and according to the maximal pool sizes that will be indicated by the preliminary experiments.

Here is the brief description of the study plans for the next 6 months:

1. MDA will complete the preliminary experiments (to provide us with a first indication on whether the HCV-Ag assay is capable of

## **II. PROJECT MANAGEMENT AND COOPERATION**

### **II. A) Managerial Issues**

During the reported period, we have experienced some set backs. Some of these are objective, global and regional issues:

1. The political tension between the Palestinian Authority and Israel, which has made it almost impossible to invite our Palestinian counterparts into Israel for planning sessions and training.
2. The war in Iraq, which has caused a state of high alert in Israel. This especially affected the availability of MDA counterparts for over two months, as they are responsible for the national blood supply in Israel.

In addition, we faced some problems when trying to set up the first study site at MDA, which were mainly due to difficulties in arranging for proper equipment for using the HCV-Ag kit. We have also had problems with establishing the HCV-Ag testing with the ORTHO kit, due to a faulty washer. This problem has been taken care of and we have already started work on the preliminary experiments.

### **II. C) Cooperation, Travel, Training and Publications**

During the reported period, the study team has held 6 planning sessions and 3 conference calls (See summary of these meetings in Appendix 1).

There were also almost daily phone calls and E-mail contacts between the counterparts, especially with EPRI where these means of communication compensated for the lack of actual face-to-face contact.

The training sessions included:

1. Training of BGU staff at MDA.
2. Training of BGU and MDA staff at MDA by a Hamilton technician after installing the new AT-win program for the automated sampler.
3. Training of MDA and BGU staff in MDA by Dover company representatives that introduced

## Appendices

### Appendix 1

#### Summary of planning sessions of the study participants

Date	BGU	MDA	EPRI	BU	Place	Topic discussed
Feb.2	*	*	* (by conf. call		MDA	The study plan ; Equipment required;
Feb. 19		*			MDA	Meeting with representatives of DOVER and Hamilton company.
Feb.23	*	*			Conf. call	Further planning
Feb.3	*				BGU	protocols
March 17	*	*			Conf. call	Study plan; manpower recruitment
April 28	*				BGU	Protocols
May 13	*	*			Conf. call	Dividing the work at each stage of the study.
June 16-17	*			*	BU	Study plan and protocols
June 25	*	*	*		MDA	Meeting with Dr. Metz – progress on the study

## Appendix 2

### Summary of training sessions

Period	Participants	Training Site	Subject of training
March 2	BGU	MDA	Pooling with the automated sampler
March 16	BGU	MDA	PCR testing
March 23 March 30	BGU, MDA	MDA	Pooling with the AT-win program on the automated sampler (given by Hamilton representative)
April 27 May 26 May 29 June 2	BGU, MDA	MDA	Using the HCV-Ag kit (given by DOVER company)