INSTITUT NATIONAL DE LA TRANSFUSION SANGUINE

# NAT versus combo Ag/Ab testing performance for reducing the HCV window phase


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# Background

- HCV Ag/Ab combos (or HCV Ag assays) an alternative for ensuring the blood safety in countries where HCV NAT cannot be introduced:

*40 to 50% of HCV-RNA pos /HCV Ab neg samples, positive with HCV combos assays*

*Laperche S, et al J. Clin Microbiol 2005*
*Laperche S, et al Transfusion 2005*
*Dean L, UK Health Protection Agency, Feb 2007*
*Tuke et al. Transfusion 2008*

**BUT**

- The cut off viremia level detected by combo assays in the window phase is unclear

- Many studies have been done in genotype 1a SC panels from the US

# Objectives

## Through an international collaboration

- Part 1 :
  Establish the differences in the sensitivity between
  HCV combo assays
  HCV antigen assays
  NAT blood screening systems
  in the detection of viremia during the WP according to the genotype and the VL by testing random subsets of the observed HCV NAT yield cases from different regions of the world.

- Part 2:
  Evaluate the analytical sensitivity of HCV combo assays and NAT systems in dilutions of high viral load HCV NAT WP samples of genotypes: 1a, 1b, 2a, 2b, 3a and 4.

# Participating laboratories

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# Part 1 : Detection of early infection

Samples : HCV NAT yield donations

Minimum 2ml with known genotype and determined VL

| Country | N samples | Genotypes |
|---|---|---|
| Egypt | 17 | 1,3,4 |
| France | 5 | 1,3,4 |
| Germany | 26 | 1,2,3,5 |
| Japan | 35 | 1,2 |
| Lithuania | 19 | 1,3 |
| Poland | 70 | 1,3,4 |
| USA | 166 | 1, 2, 3, 4 |
| TOTAL | 338 | |

# Part 1 : Detection of early infection

## Assays

- HCV Combos (INTS)
  - Monolisa HCV Ag/Ab Ultra (BioRad)
  - Murex HCV Ag/Ab Combination (Abbott)
- HCV Ag (PEI)
  - Architect HCV Ag (Abbott)

# Part 1 : Detection of early infection

## Method

HCV Combos (INTS)

- Monolisa HCV Ag/Ab Ultra (BioRad)
- Murex HCV Ag/Ab Combination (Abbott)

| | | Combo 2 | Single testing | |
|---|---|---|---|---|
| | Result | Neg | 0.9<S/CO <2 | >or = 2 |
| Combo 1 Single testing | Neg | **NEGATIVE** | *Testing in duplicate* | *Testing in duplicate* |
| | 0.9<S/CO <2 | *Testing in duplicate* | *Testing in duplicate* | *Testing in duplicate* |
| | >or =2 | *Testing in duplicate* | *Testing in duplicate* | **POSITIVE** |

**S/CO Mean value**

# Part 2 : Analytical sensitivity

## Samples

- Dilution panel (DDL)

Samples used for preparation of HCV dilution panels:

| Panel number | origin | gen | IU/ml |
|---|---|---|---|
| P01 | Egypt | 1a | 6,26E+05 |
| P02 | Egypt | 1a | 1,17E+06 |
| P03 | Japan | 1b | 6,90E+07 |
| P04 | Japan | 1b | 2,90E+07 |
| P05 | Japan | 2a | 1,70E+07 |
| P06 | Japan | 2a | 4,90E+07 |
| P07 | Japan | 2b | 7,40E+06 |
| P08 | Japan | 2b | 1,60E+06 |
| P09 | Lithuania | 3a | 3,00E+06 |
| P10 | Lithuania | 3a | 2,00E+07 |
| P11 | Egypt | 4 | 1,08E+06 |

**Panel 1 :**
half log dilutions from $3x10^7$ to $3x10^5$IU/ml

**Panel 2 :**
half log dilutions from $1x10^5$ to $3x10^3$IU/ml

**Panel 3 :**
half log dilutions from $1x10^3$ to $10^{-1}$IU/ml

- HCV RNA WHO, Eurohep and Hiroshima chimpanzee infectivity standards

# Part 2 : Analytical sensitivity

## Method (1)

- Panel 1 in duplicate
  - Monolisa HCV Ag/Ab Ultra (BioRad) (INTS)
  - Murex HCV Ag/Ab Combination (Abbott) (INTS)
  - Architect HCV Ag (Abbott) (PEI)

➢ *Cut off level of combos for each genotype*

- Panel 2 + standards in duplicate
  - Combos (INTS)
  - Architect HCV Ag if the last dilution sample of panel 1 positive (PEI)
  - bDNA (Berkeley, USA)
  - Cobas Taq Man (Roche Molecular Systems) (INTS)

➢ *Cut off level of combos for each genotype cross VL calibration*

- Panel 3 + reference standards in 12 replicates
  - Procleix Ultrio on Tigris IDT (Poland)
  - Taq Screen MPX on S201 system IDT (PEI)

➢ *Cut off level of NAT for each genotype*

# Part 2 : Analytical sensitivity

## Method (2)

- The cut off crossing point of combo ELISA expressed as IU/ml or copies/ml will be determined by regression analysis after logit log transformation of the response curves. The cut off will be compared with the 95% and 50% detection limits found with NAT screening systems and will be expressed in IUs and copies/ml according to calibration in the bDNA 3.0 assays as the reference method and the Roche TaqMan assay for comparison.

- Assuming that for each genotype the viral doubling time of HCV is comparable (0.45 days) the time difference between the 50% hit rate seroconversion point for Ultrio ID, Ultrio MP-NAT (1:8) and TaqScreen MP-NAT (1:6), the HCV Ag and combo ELISA cut off crossing points will be extrapolated for each genotype from the results of the dilution panels.

# Preliminary results

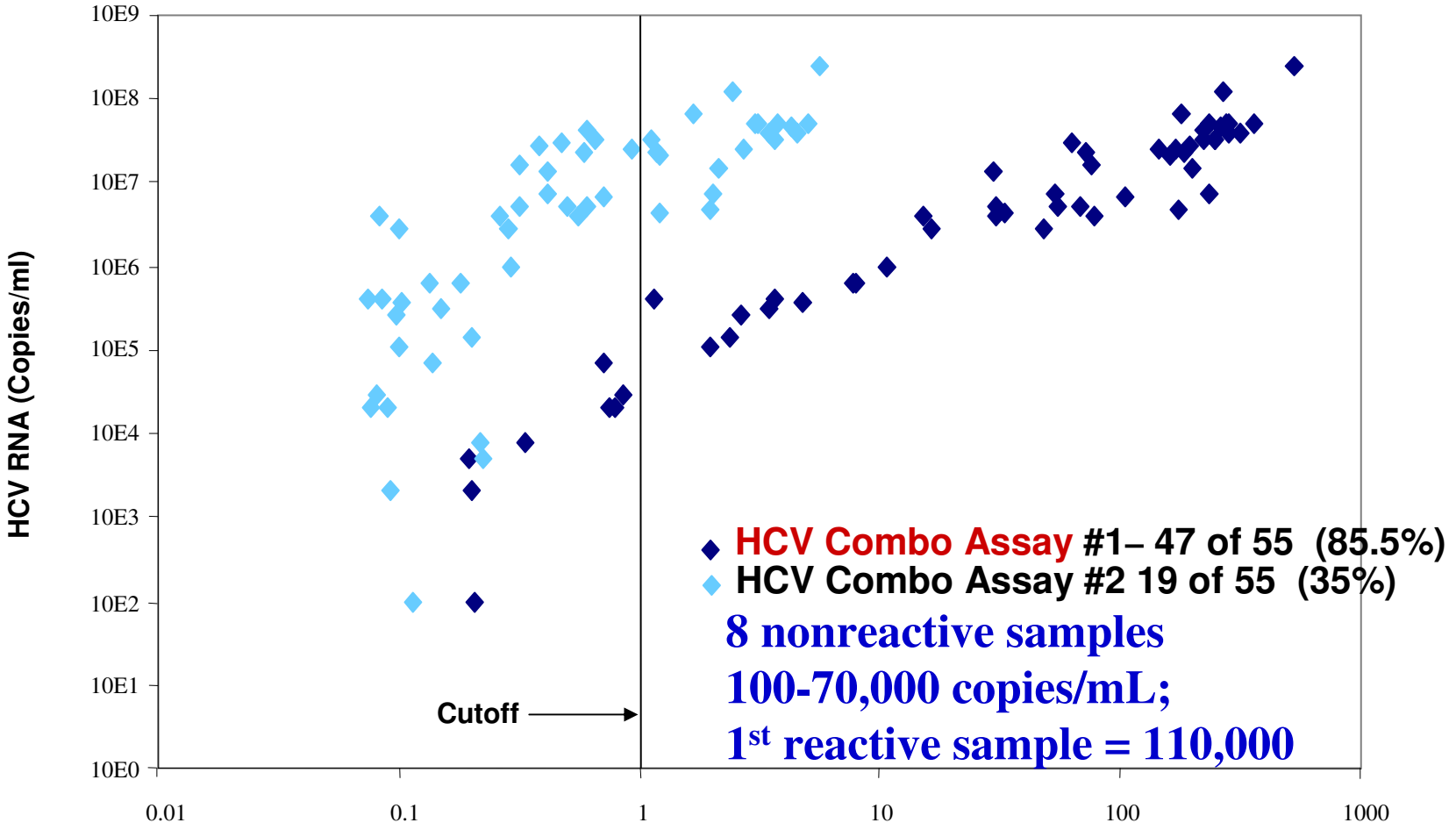
| Country | N | Monolisa screening *<br>N positive |
|---|---|---|
| Egypt | 17 | 6 (35.3%) |
| France | 5 | 1 (20%) |
| Germany | 26 | 12 (46%) |
| Japan | 35 | 22 (63%) |
| Lithuania | 19 | 3 (15.8%) |
| Poland | 70 | 19 (27.1%) |
| USA | 166 | 67 (40.4%) |
| TOTAL | 338 | 130 (38.5%) |

* Including grey zone (0.9-1), to be confirmed by repeat testing for some samples

# ARC Study (2006) of Monolisa HCV Ag/Ab Ultra (BioRad)

| HCV Sample Type | No. Reactive | | Viral Loads (Copies/mL) |
|---|---|---|---|
| | 1.0 cutoff | 0.5 cutoff | |
| 17 RNA +/Ab - | 7 (41%)* | 8 (47%)* | Rx = 4.3-50x10^6 +/- = 640,000 NonRx = 18,000-333,000 (1 @ 2.5x10^6) |
| 50 RNA +/Ab + | 50 | 50 | >10^6 |
| 20 RNA -/Ab - | 0 | 0 | NA |

# Evaluation of HCV NAT Positive/Anti-HCV Antibody Negative Samples - samples identified by MP-NAT in blood donors from U.S.

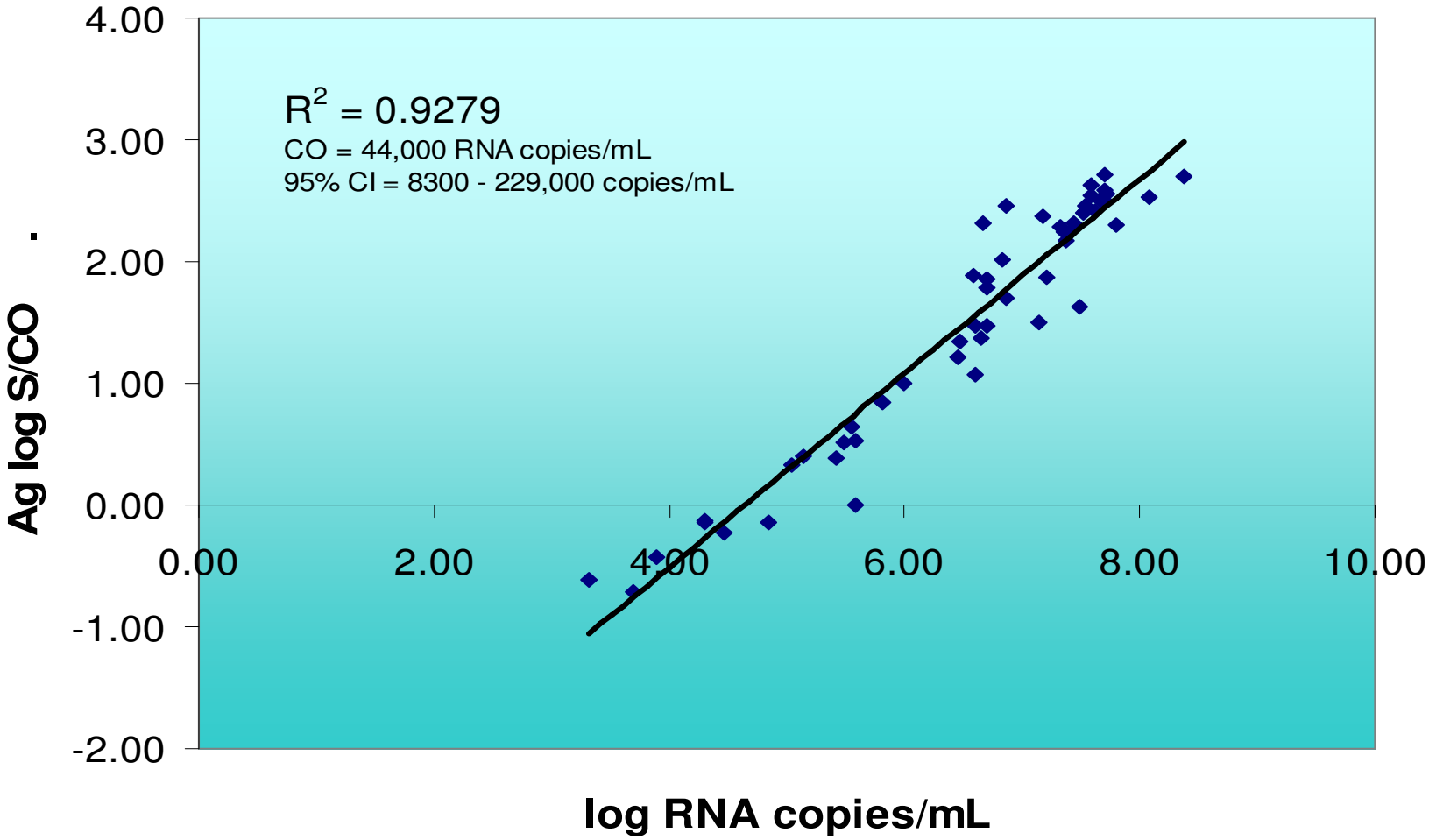





Abbott
A Promise for Life

# Research HCV Combo Assay

## Correlation with HCV RNA titer



# Perspectives

1. Include additional samples to constitute an international panel of HCV NAT Yield samples
2. Establish cost effectiveness ratio of each method
3. Extend the study to HIV NAT yield samples

# Sponsorship

- Abbott providing
Murex HCV Ag/ Ab Combination and Architect Ag assays.

- Biorad providing
Monolisa HCV combo Ag/Ab Ultra assays

- CHIRON-Novartis providing
Ultrio and Procleix assays
a grant to INTS for the study
a support for the preparation and shipment of samples