EVALUATION OF A NOVEL PLATFORM FOR DETERMINING GENOTYPES OF HCV*

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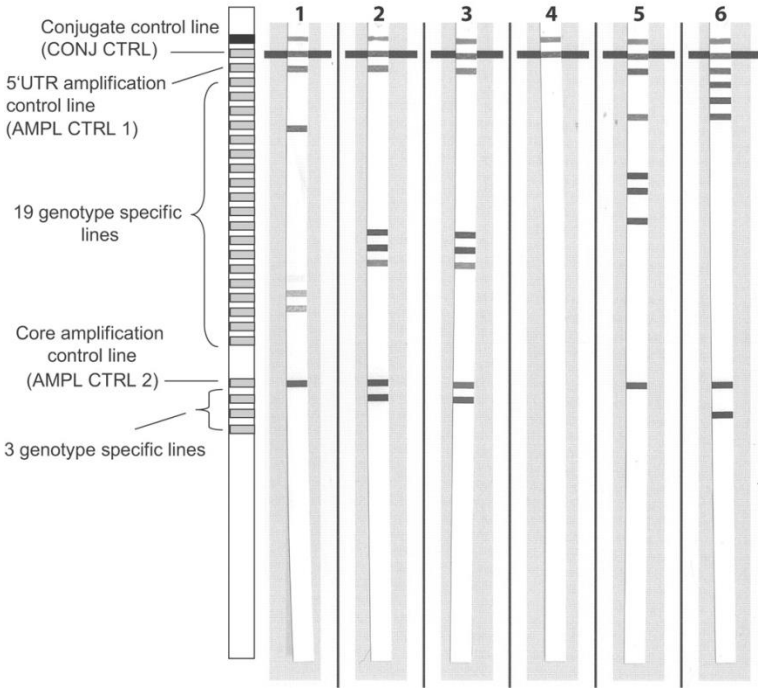
*Presented at Association of Molecular Pathology Annual Meeting, Nov 5th-7th, Austin, TX

HCV Genotyping: Significance and Challenges

- Key parameter in evaluation of HCV infected persons
  - Genotype has significance in therapeutic decision-making/prognosis
  - Likely to remain of considerable utility for at least medium term
  - Confirmation of genotype typically required pre-treatment (payment)
  - Marked increase in test utilization since DAA introduced
- Balancing cost/efficiency of testing vs accuracy
  - Viral heterogeneity presents challenges in accurate identification
  - Using more conserved regions (simplicity of testing constructs)
  - Using more variable regions (accuracy of identification)

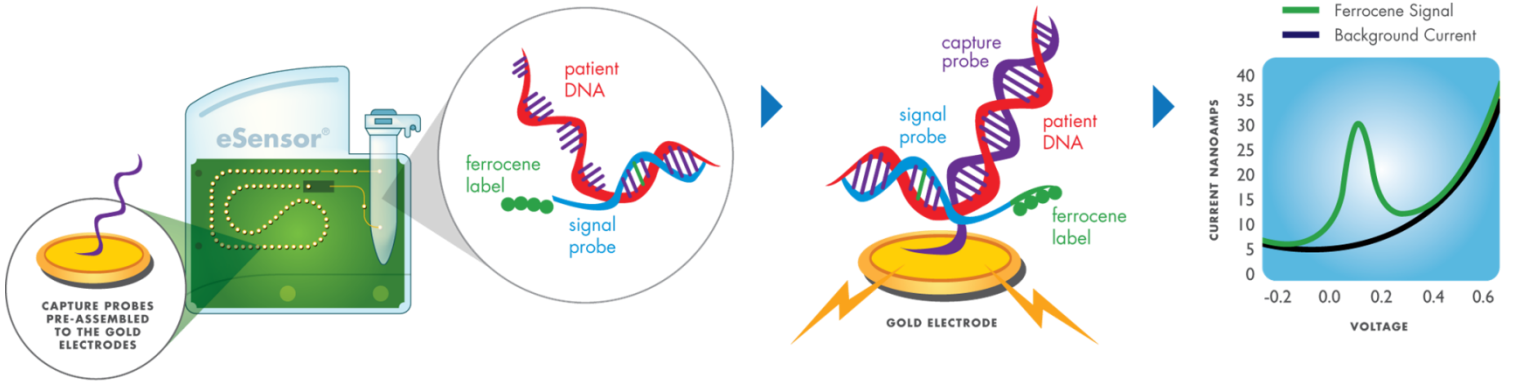
HCV GENOTYPING: HCV GENOTYPE V 2.0 (LIPA)

- **Effectively the 'gold standard' in routine laboratory testing**



- Limited throughput, several manual operations, significant liquid waste volume
- Subjective reader-dependent interpretation of 'difficult' patterns
- Overall success rate to subtype with unaltered interpretation guideline 93-95%

HCV GENOTYPING: E-SENSOR® HCVG DIRECT*



1 The target DNA is mixed with the signal probe solution. If the applicable target DNA is present, hybridization to the signal probes occurs immediately.

2 The solution is pumped through the cartridge's microfluidic chamber and the target DNA/signal probe complex completes the reaction with the pre-assembled capture probe.

3 Voltage is swept across each electrode and target DNA is analyzed by electrochemical detection.

**Manufactured by GenMark Dx, Carlsbad, CA*

Clinical Performance Study

- Goal was to stringently compare performance against current standard
- Sample set consisted of de-identified clinical samples (stored at -70°C)
- All samples previously analyzed by LiPA at LabCorp CET
- Total of 437 samples included in the final dataset divided into 2 cohorts
- Cohort designation based on LiPA result (genotype, subtype, pattern)
- LiPA definitively genotyped/subtyped (Cohort A)
- LiPA inconclusive/incomplete (Cohort B)

Clinical Study (Cohort A)

- Samples (n=269) yielded definitive results by LiPA
- Genotypically balanced cohort (35-40 samples per category*)
- Sample set biased to include typical and atypical LiPA patterns
- NS5B sequencing only performed on discordant samples

**Only 5 samples were available that had been identified as genotype 5*

Clinical Study (Cohort B)

- Samples (n=168) yielded incomplete/problematic results by LiPA
  - Indeterminate (banding pattern not consistent with recognized pattern)
  - Highly atypical banding patterns (typically called to genotype despite lacking key band(s))
  - Genotype 1 no subtype designation
  - Genotype 2 no subtype designation
  - No core bands present result in an ambiguous result (1 possible 6 or 1 possible 4)
  - Identified as a mixture of two genotypes
- 3-5% of routine clinical samples fall into this category
- NS5B sequencing performed on all samples to generate reference result

RESULTS: COHORT A

		LiPA				HCVg			
Resolved Type	n	Correct	Discordant	No subtype	No call	Correct	Discordant	No subtype	No call
1a	42	39	3	0	0	41	0	0	1
1b	41	40	1	0	0	40	0	0	1
2a/c	30	30	0	0	0	30	0	0	0
2b	42	42	0	0	0	42	0	0	0
3	39	39	0	N/A	0	38	0	N/A	1
4	36	36	0	N/A	0	36	0	N/A	0
5	5	5	0	N/A	0	5	0	N/A	0
6	34	34	0	N/A	0	31	0	N/A	3
TOTAL	**269**	**265**	**4**	**0**	**0**	**263**	**0**	**0**	**6**

- LiPA correctly called **265/269 (98.5%)** to genotype/subtype
- HCVg correctly called **263/264 (99.6%)** of samples to genotype/subtype**; 4** no-calls

- **All 4 LiPA erroneous calls were genotype 1 viruses called as genotype 5**
- **2/6 HCVg no-calls were genotype 1 viruses called as 5 by LiPA**
- **3/6 HCVg no-calls were genotype 6 viruses (6h, 6n, 6q)**
- **1/6 HCVg no-calls was a genotype 3 virus (3a)**

RESULTS: COHORT B

		HCVg			
Genotype	n	Correct	Discordant*	No subtype	No call
1¤	2	0	2 (2)	N/A	0
1a	52	43	8 (7)	0	1
1b	20	11	9 (3)	0	0
2¥	1	0	1	N/A	0
2a/c	13	7	3 (0)	3	0
2b	43	28	13 (12)	1	1
3	17	3	7	N/A	7
4	4	3	0	N/A	1
6	8	7	0	N/A	1
Mixed	8	6	2	N/A	0
TOTAL	**168**	**108**	**43 (24)**	**4**	**11**

*Numbers in parentheses indicate discordant at the genotype level including erroneous mixed calls
¤ Genotype 1c (1) and 1g (1)
¥ Genotype 2j (1)

- HCVg correctly called **108/157 (68.8%)** of samples to subtype; **131/157 (83.4%)** to genotype; **11** no-calls
- Both assay systems overcalled 'mixed' genotypes **(LiPA n=53; HCVg n=24; NS5B n=8)**
- HCVg markedly improved resolution of 'problematic' genotype 1 samples **(54/74)**

Conclusions

- eSensor HCVg assay accurately/efficiently determines HCV genotypes
  - Resolves majority of LiPA untypable samples (approx 70%)
  - Readily automatable, expandable system
  - Objective determination of results
  - Highly dependable instrumentation; minimal maintenance
- Experience in the laboratory since implementation
  - 99.5% samples generate a definitive result (evaluation of no-call samples underway)
  - <0.2% cartridge failure rate
  - Significant decrease in labor utilization and in time to result

LabCorp
Laboratory Corporation of America

……Questions???