Validation of a point-of-care quantitative HCV-RNA test using capillary blood from the finger (Xpert® HCV Viral Load, Cepheid®)

Kantonsspital Aarau

Andrea Bregenzer[1], Nicole Warmann[1], Cornelia Ottiger[2], Christoph Andreas Fux[1]

[1]Department of Infectious Diseases and Hospital Hygiene, Cantonal Hospital Aarau,
[2]Institute for Laboratory Medicine, Cantonal Hospital Aarau

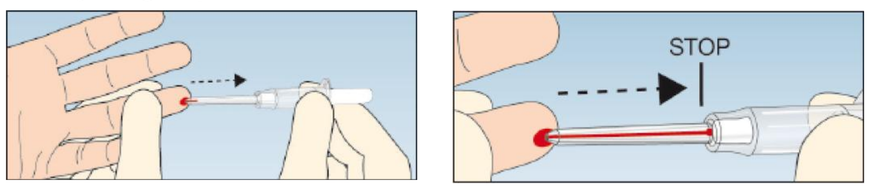
AIM

- To compare point-of-care quantitative HCV-RNA measurement using 100µl capillary blood from the finger (Xpert® HCV Viral Load, Cepheid®) with the standard quantitative HCV-PCR test Cobas® performed in the laboratory with 1ml venous EDTA-plasma.

METHODS

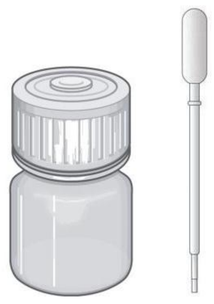
- Since 11/2016, patients of our Infectious Diseases Outpatient Clinic with an indication for HCV-RNA determination and written informed consent provided both venous and capillary blood for quantitative HCV-RNA measurement.
- Venous plasma was tested using Cobas® (quantification limit: 15 U/ml; result within 1 week).
- 100µl capillary blood collected from the finger with an EDTA-minivette was immediately transferred into an Xpert® HCV Viral Load cartridge (quantification limit: 10 U/ml; result within 105 minutes).
- The addition of 1ml Cepheid® blood sample diluent and the use of whole blood consisting of ~50% plasma resulted in a ~1:20 dilution compared to the Cobas® plasma sample.

1) Collect 100µl capilary blood by finger-prick into an EDTA-minivette



2) Load blood immediately into Xpert® HCV Viral Load cartridge

3) Add 1ml dilution buffer (provided by Cepheid®)



4) Put cartridge into GeneXpert®

5) Result available after 105min

RESULTS

- Of 57 samples (from 42 patients), 32 (56.1%) tested positive with Cobas® (11 genotype (gt) 1; 10 gt 3; 11 gt 4).
- Sensitivity of the Xpert® assay using capillary blood was 93.8% (30/32) and specificity 96.0% (24/25) (PPV: 96.8% (30/31); NPV: 92.3% (24/26)).
- The 2 Cobas®-positive samples missed by Xpert® had low viral load (68 U/ml and <15 U/ml) and were both gt 4 one week after DAA (direct acting antiviral) start.
- The sample which was positive with Xpert® (HCV detected <10 U/ml), but not detected with Cobas® (<15 U/ml) was gt 3 four weeks after DAA start.
- Excluding two extreme outliers considered as technical failures, 23 samples (9 gt1; 7 gt 3; 7 gt 4) with quantitative results in both tests remained for the correlation analysis (HCV-RNA range with Cobas®: 3780-10.2 Mio. U/ml = 3.58-7.01 log U/ml).
- On average, Cobas® results were 21.1-times or 1.3 log higher, which corresponds to the dilution factor.
- Cobas® and Xpert® values were highly correlated (R^2=0.91).
- The equation for the fitted regression line was "Cobas®(log U/ml)=1.0291*Xpert®(log U/ml)+1.1318".

LITERATURE

Grebely J, et al. Lancet Gastroenterol Hepatol 2017;2(7):514-520

Qualitative performance of the Xpert® HCV Viral Load test with 100µl finger-prick capillary blood

		Cobas® (venous plasma) (Gold standard)		
		Positive	**Negative**	**Total**
Xpert® HCV Viral Load (capillary blood)	**Positive**	(a) 30	(b) 1	**31**
	Negative	(c) 2	(d) 24	**26**
	Total	**32**	**25**	**57**

Prevalence = (a+c)/(a+b+c+d) = 32/57 = **56.1%** (95% CI: 43.3-68.2%)
Concordant result = (a+d)/(a+b+c+d) = 54/57 = **94.7%** (95% CI: 85.6-98.2%)

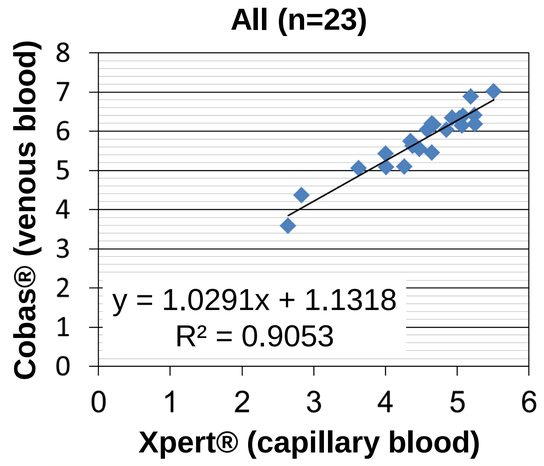
Sensitivity = a/(a+c) = 30/32 = **93.8%** (95% CI: 79.9-98.3%)
(proportion of HCV-RNA-positives correctly identified by the capillary test)
Specificity = d/(b+d) = 24/25 =**96.0%** (95% CI: 80.5-99.3%)
(proportion of HCV-RNA-positives correctly identified by the capillary test)
PPV (positive predictive value) = a/(a+b) = 30/31 = **96.8%** (95% CI: 83.8-99.4%)
(proportion of individuals with a positive capillary test result actually being HCV-RNA-positive)
NPV (negative predictive value) = d/(c+d) = 24/26 = **92.3%** (95% CI: 75.9-97.9%)
(proportion of individuals with a negative capillary test result actually being HCV-RNA-negative)

Performance of the Xpert® HCV Viral Load test with 100µl finger-prick capillary blood 1-4 weeks after DAA-start (discordant results in red)

Pat.	HCV-Geno-type	Weeks after DAA start	Cobas® (venous plasma)		Xpert® HCV Viral Load (capillary blood; ~1:20 dilution)	
			U/ml	Log U/ml	U/ml	Log U/ml
1	1a	1	44	1.64	HCV detected <10	HCV detected <1.00
2	1a	2	16	1.2	HCV detected <10	HCV detected <1.00
3	3	1	26	1.41	HCV detected <10	HCV detected <1.00
4	3	2	81	1.91	HCV detected <10	HCV detected <1.00
4	3	4	Not detected (<15)	Not detected (<1.2)	HCV detected <10	HCV detected <1.00
5	4	1	68	1.83	Not detected (<10)	Not detected (<1.00)
6	4	1	HCV detected <15	HCV detected <1.2	Not detected (<10)	Not detected (<1.00)
7	4	1	23	1.36	HCV detected <10	HCV detected <1.00

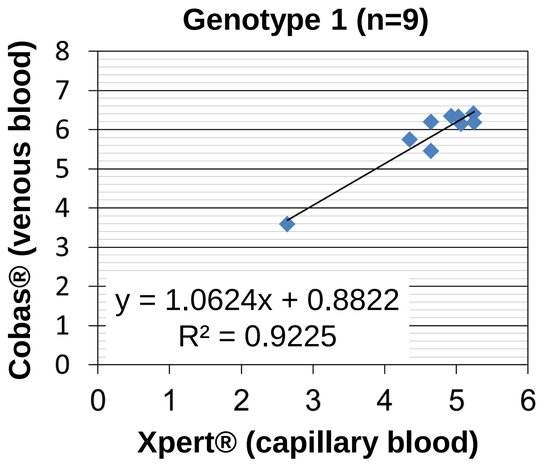
Correlation of quantitative HCV-RNA results (log U/ml)

All (n=23)



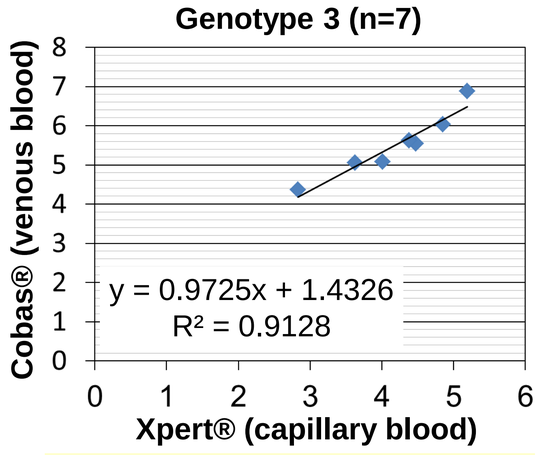
Range Cobas®: 3.58-7.01 log U/ml (3780-10.2 Mio U/ml)

Genotype 1 (n=9)



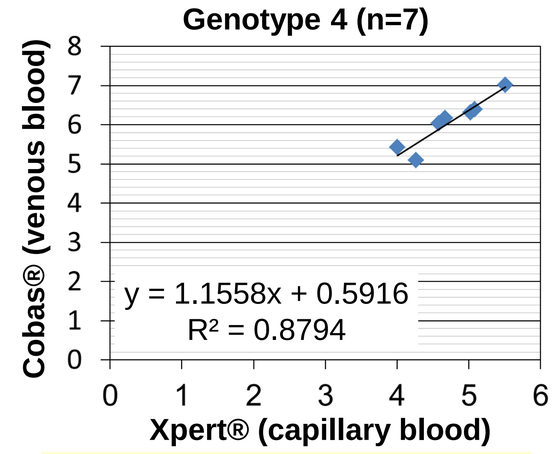
Range Cobas®: 3.58-6.41 log U/ml (3780-2,573,715 U/ml)

Genotype 3 (n=7)



Range Cobas®: 4.36-6.88 log U/ml (22,752-7,665,565 U/ml)

Genotype 4 (n=7)



Range Cobas®: 5.09-7.01 log U/ml (122,635-10.2 Mio U/ml)

CONCLUSIONS

- Point-of-care quantitative HCV-RNA measurement with capillary blood from the finger is a convenient, rapid and sufficiently reliable method to further evaluate HCV-antibody-positives, monitor HCV treatment response and detect HCV-re-infection.
- For patients with obliterated peripheral veins after long-term intravenous drug use, it removes a crucial barrier to HCV treatment and re-infection monitoring.
- Same-day results might improve linkage to care.

ACKNOWLEDGEMENT/SPONSORING

- We thank the Kirby Institute, Australia (Francois Lamoury, Tanya Applegate and Gregory Dore) for providing us with the protocol for the Xpert® HCV Viral Load test with capillary blood.
- Axonlab® loaned us a GeneXpert IV free of charge.
- Cepheid® provided us with free lancets, EDTA-minivettes and dilution buffer.
- Xpert® HCV Viral Load tests were sponsored by Gilead®.