Medicines & Healthcare products Regulatory Agency

HCV NAT assays and the challenge of genetic variants in drug therapy

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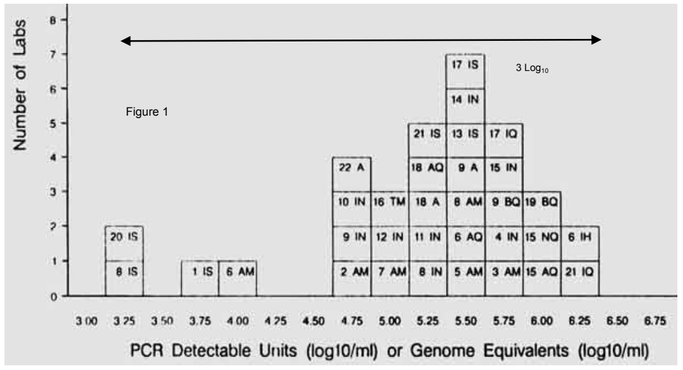
NIBSC Confidence in Biological Medicines

BACKGROUND

The use of nucleic acid-based techniques for the detection of HCV RNA has been standard practice since the mid 1990's. Such techniques facilitate the accurate and sensitive detection of HCV. However, the determined sensitivity of an assay is only a good as the initial calibration. The use of an international primary calibrator allows assay manufactures and laboratories to calibrate their assays against the same material allowing harmonisation across all assays systems.

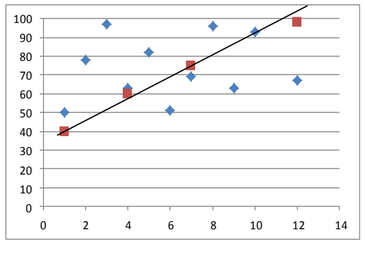
NIBSC work with the WHO to produce these reference materials. Figure 1 shows the variation in data received from laboratories that took part in the study to establish the 1st international standard for HCV RNA in 1996. There is over a 3Log$_{10}$ inter-lab variability.



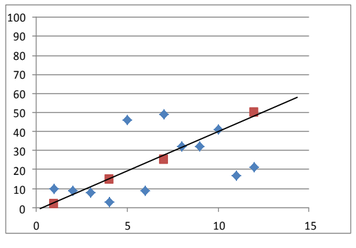
Principles of producing international standards for NAT diagnostic assays are:

- Controls all steps of the assay
- Behaves in similar way to clinical material
- Material is stable long term
- Material is assigned arbitrary units value following multicenter collaborative study
- Data is submitted to WHO ECBS for establishment

Complex highly sensitive assay



Less sensitive, point of care test



Using International Standards allows the amount of specific material in an unknown sample to be determined as a relative potency. This enables data from assays with different performance characteristics to be compared as demonstrated in the schematic above.

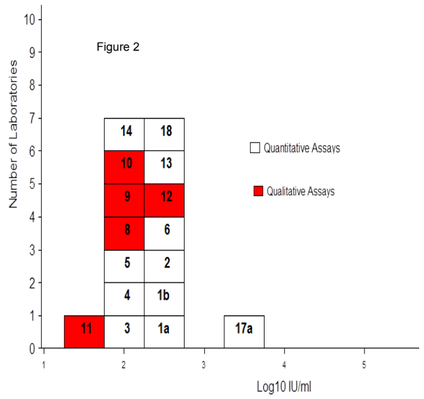
USE OF HCV RNA WORKING REAGENTS

In addition to accurate assay calibration, it is important to monitor each assay run. Inclusion of a run control, EXTERNAL to assay controls, placed in every test permits regular monitoring of data for trends that indicate:

- Lot to lot variation in kit performance
- Early degradation of kit components
- Early evidence of equipment failure
- Variable operator performance

In a collaborative study to establish the 5th HCV RNA international standard, a low titre external run control was also included for calibration. This material is intended to assure the quality of assay performance by including it in each assay run.

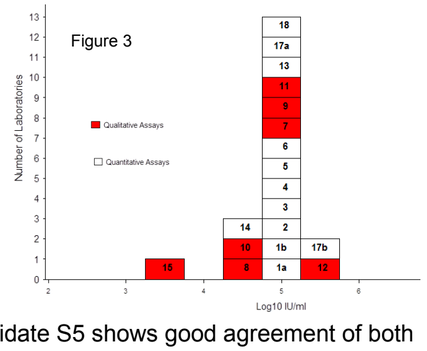
Figure 2 shows the data set reported in IU/ml. The reagent contains 2.25 Log$_{10}$IU/ml (±0.8 Log10)



WHO HCV INTERNATIONAL STANDARDS

An international standard has been available since 1996, the standard comprises genotype 1a and has been fundamental in the standardisation of HCV RNA assays. To establish the 5th HCV RNA standard 6 candidates were assessed in an international collaborative study. 17 laboratories returned data, 12 in quantitative format from a range of assays as shown in the table below.

Assay type	S1	S2	S3	S4	S5	S6
Roche CAP/CTM v2	4.94	4.13	5.04	5.65	5.06	2.37
Roche COBAS 6800	5.11	4.03	5.14	5.66	5.28	2.73
Versant kPCR HCV RNA	5.27	4.47	5.06	5.80	5.07	2.38
Artus HCV QS-RGO Kit V1	5.04	4.10	5.26	5.65	5.05	1.87
Abbott real time HCV (0.5mL)	5.02	4.38	4.99	5.50	4.87	2.05
Roche COBAS 6800	5.15	4.48	5.04	5.48	5.10	2.17
Roche CAP/CTM v2	5.20	4.60	5.10	5.70	5.13	2.48
Qiagen QiaSymphony	4.96	4.32	4.98	5.48	5.18	2.33
Roche CAP/CTM v2	4.48	4.32	4.95	5.42	4.60	2.13
In house	5.56	4.98	5.68	6.09	5.72	3.40
In house	4.83	4.31	5.06	5.77	5.47	
Roche CAP/CTM v2	5.39	4.55	5.22	5.46	5.35	2.59



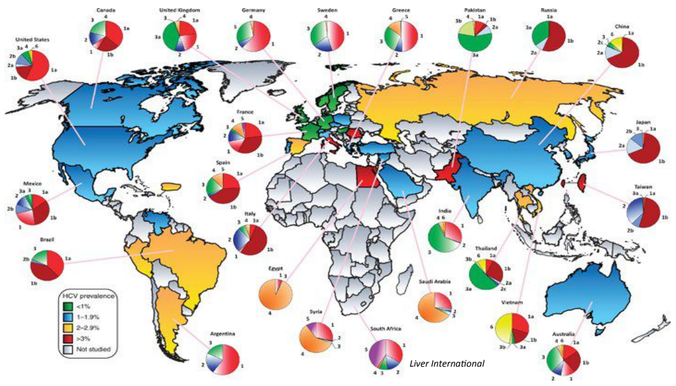
Relative potency assignment of candidate S5 shows good agreement of both quantitative and qualitative assays is (figure 3). Candidate S5, a window period plasma donation of genotype 1a, has been established as the 5th international standard for HCV RNA with a unit of 5.0 Log$_{10}$ IU/mL when reconstituted in 1.1ml . However, global prevalence of different genotypes prompts for further reference materials to ensure assays are optimised.

DRUG THERAPY AND VARIANT DETECTION

The global prevalence of HCV genotypes varies from region to region as shown in figure 4. It is known that not all assays are equally sensitive when assaying the 6 major HCV genotypes.

A study showed that the results obtained for genotypes 1, 2 and 3 were identified in 100% of cases, the results for genotypes 4, 5 and 6 were less satisfactory, with positive samples being missed by some assays. (*High proficiency in detecting the six major hepatitis C virus genotypes of laboratories involved in testing plasma by nucleic acid amplification technology G. Gentili, G. Pisani, J. Saldanha, K. Cristiano, M. Wirz, G. M. Bisso, C. Mele and the EQA Participants Vox Sanguinis (2003) 85 , 114–116)*

Figure 4: Global prevalence of HCV genotypes



As detection of all HCV genotypes is critical for laboratories involved in testing plasma for HCV and for all blood donation centres, NIBSC prepared a panel with all six major genotypes of HCV.

Treatment of HCV depends upon which HCV genotype the patient presents. The specific HCV genotype is also important as certain patients infected with HCV genotype 1a of occasion have higher rates of virological failure than those infected with HCV genotype 1b. The NIBSC HCV genotype panel contains HCV genotypes 1a and 1b which allows laboratories to assess whether their assays can discriminate these two genotypes.

CONCLUSIONS

The results generated from one assay are not comparable with another assay unless they have been calibrated against a common standard.

The availability of an international standard has been proven to be fundamental in the standardisation of different assay systems

Accurate detection of all HCV genotypes in vital in the provision of a safe blood supply.

For further information regarding different control materials available from NIBSC please visit www.nibsc.org.