QIAGEN-7th DIAGNOSTIC DAYS

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HCV TESTING FOR TRIPLE THERAPY

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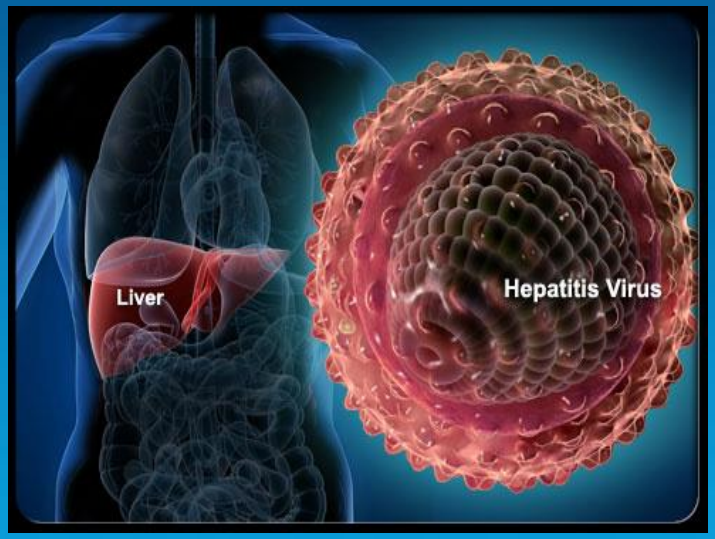
QIAGEN would like to thank our speaker, Dr. Samir Dervisovic, for his presentation.

OVERVIEW

- Hepatitis C virus
- Epidemiology of HCV in the United Kingdom
- Therapy for HCV:
  - -standard of care treatment
  - -new therapies
  - -triple therapy
- Monitoring of treatment
- Norfolk and Norwich University Hospital experience

HEPATITIS C VIRUS

- 1989 Michael Houghton's laboratory at Chiron Corporation and Daniel Bradley's laboratory at CDC identified the virus



- Family Flaviviridae, genus Hepacivirus

- Roughly spherical, enveloped virus 55 nm in diameter
- Genome: positive-sense, single-stranded RNA virus, 9.6 kb in length
- Genome contains a large ORF encoding a single polyprotein (~3010 amino acids)

HCV REPLICATION AND GENETIC DIVERSITY

- $1x10^{12}$ virions are produced daily in a chronically infected human
- Absence of proofreading by the NS5B RNA polymerase and “tolerance” of many genomic regions for multiple nucleotides results in the relatively rapid accumulation of viral mutations
- As a result, HCV exists in each infected person as a “swarm”(quasispecies) of closely related but distinct genetic sequences

HCV GENETIC DIVERSITY

- 7 HCV genotypes may have less than 50% nucleotide sequence identity (Mandell, Douglas, and Bennett's: Principles and Practice of Infectious Diseases, 7th Edition, 2010)
- Within individual genotypes , strains could be further grouped into subtypes sharing 75-85% nucleotide sequence identity within regions of the genome
- Major genotypes started diverging ~300 years ago
- Main genotype in the West: G1 and G3

HEPATITIS C DISEASE BURDEN IN THE UK

- Most common blood-borne viral infection in the UK
- National estimates suggest that around 216,000 individuals are chronically infected with hepatitis C, 0.5% prevalence. (Hep C Audit 2013;1-13)
- Laboratory-confirmed new diagnoses reported in England rose to 10,873 cases in 2012 (PHE Report July 2013)
- Statistical modelling predicts that 15,840 individuals will be living with HCV-related cirrhosis or HCC in England in 2020 if left untreated (HPA, Hepatitis C in the UK:2012 report)

HEPATITIS C DISEASE BURDEN IN THE UK

- Liver cirrhosis: within 20–30 years
- 10-20% of chronically infected individuals will develop cirrhosis
- 1–5% may develop hepatocellular carcinoma
- 1979 hospital admissions due to HCV in 2010*
- 323 deaths in 2010 attributable to chronic HCV*
- In UK **only 3%** of people chronically infected with HCV are receiving treatment each year

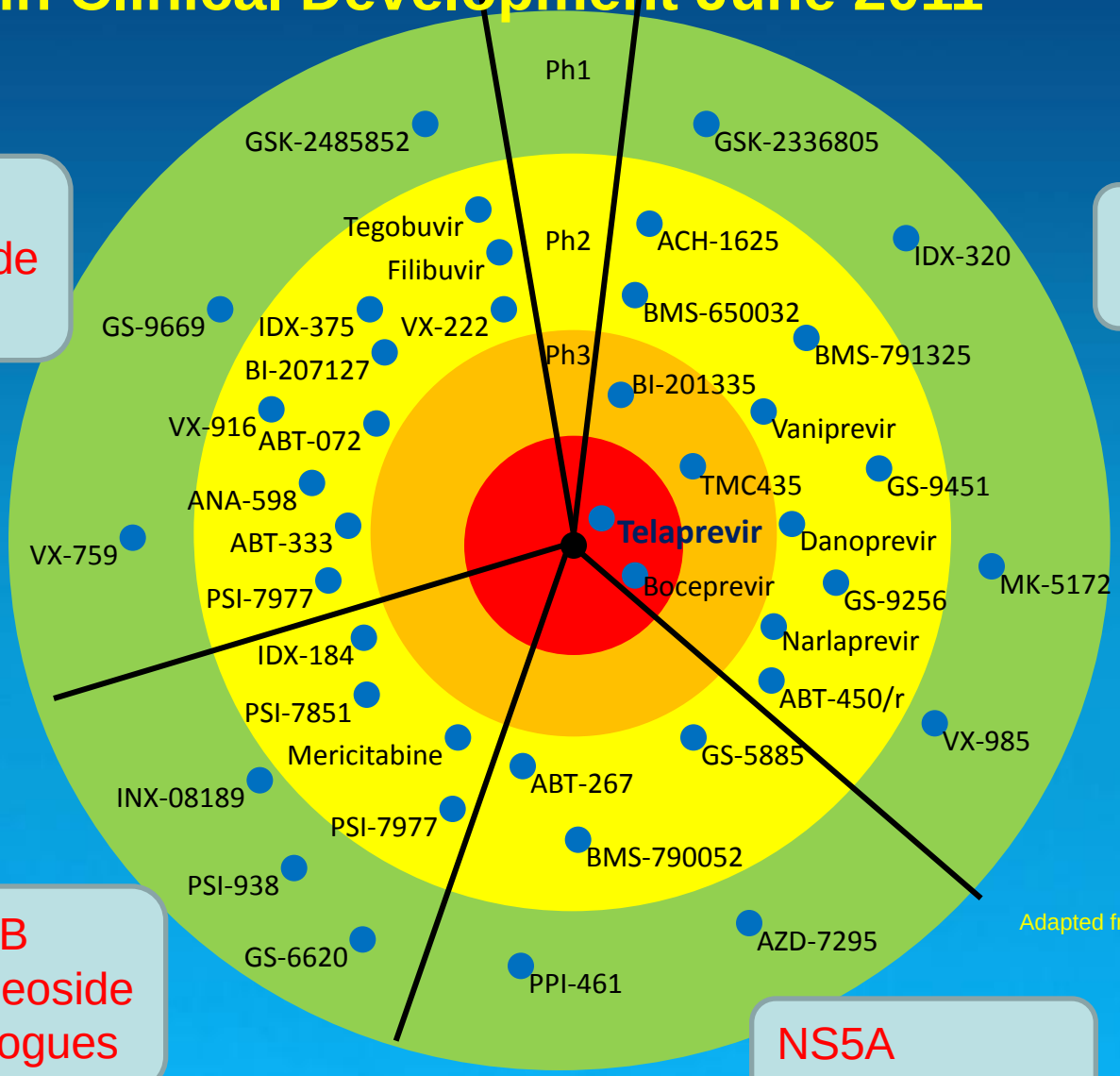
*(HPA, Hepatitis C in the UK:2012 report)

TREATMENT FOR HEPATITIS C

Treatment	Cure rate
1991-Interferon	5%
1998-2003 Peg-IFN+RBV	25%
2011 Peg-IFN+RBV+PI	55-75%
2013 multiple drugs (57)	?>90%

(interferons, cyclophilin inhibitor, NAPI, NNPI, new generation PI, NS5AI)

HCV DAAs in Clinical Development June 2011



NS5B Non-nucleoside inhibitors

NS3 Protease Inhibitors

NS5B Nucleoside analogues

NS5A Inhibitors

Adapted from: Schlűtter, J., Nature. 2011 Jun 8;474(7350):S5-7

Our Vision
To provide every patient with the care we want for those we love the most

Norfolk and Norwich University Hospitals NHS
NHS Foundation Trust

PROTEASE INHIBITORS

- 1st generation: Telaprevir and Boceprevir
- Significant issues: -trice daily dosing
  -toxicity
  -low genetic barrier
  -extensive drug-to-drug interactions

- 2nd wave: Simeprevir, Asunaprevir, Danoprevir, Sovaprevir, ABT-450r, Vaniprevir, Faldaprevir

- Issues: -same or similar genotype coverage
  -same resistance profile

- 2nd generation: MK-5172, ACH-2684
- Issues: -broader genotype coverage
  -active against variants carrying R155K mutation

NEW ERA OF TREATMENT FOR HCV INFECTION

'New standard of care': Response Guided Therapy (RGT)

- RGT=treatment algorithm individualizing treatment based on *virological* response
- Goals of Response Guided Therapy:

a) Shorten therapy if possible in those who exhibit favourable viral kinetics

b) Identify subjects who are unlikely to have a response

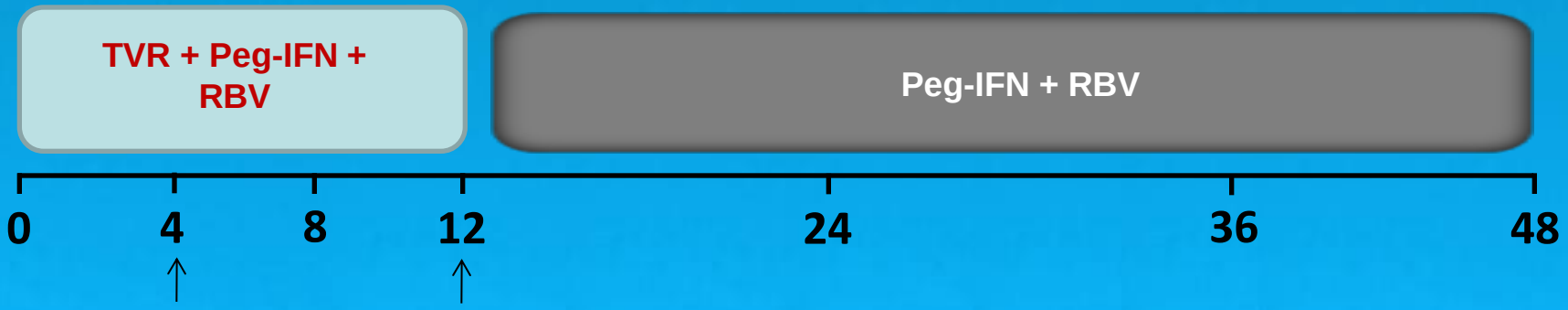
c) Limit side effects

d) Cost

TELAPREVIR TREATMENT IN G1 PATIENTS

- Prior relapser and treatment naïve patients

- Prior partial and null responder and cirrhotic patients



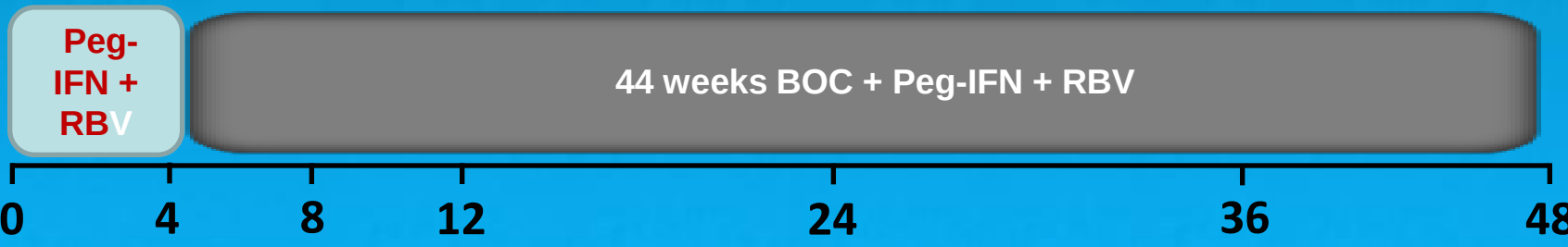
Telaprevir SmPC.

BOCEPREVIR TREATMENT IN G1 PATIENTS

- **Treatment naïve early responders** — week 8 and 24 undetectable HCV RNA

Treatment naive late responders and treatment experienced — week 8 detectable and week 24 undetectable HCV

- **Cirrhotic patients and null responders**



Boceprevir SmPC.

TRIPLE THERAPY STOPPING RULES

Time Point	Criteria	Stopping Rule
Telaprevir		
Wk 4 or 12	HCV RNA > 1000 IU/mL	Discontinue all therapy
Wk 24	Detectable HCV RNA	Discontinue Peg-IFN+RBV
Any	Discontinuation of Peg-IFN+RBV for any reason	Discontinue TVR also
Boceprevir		
Wk 12	HCV RNA ≥ 100 IU/mL	Discontinue all therapy
Wk 24	Detectable HCV RNA	Discontinue all therapy
Any	Discontinuation of Peg-IFN+RBV for any reason	Discontinue BOC also

Telaprevir [package insert]. May 2011. Boceprevir [package insert]. May 2011.

IMPORTANCE OF HCV RNA PCR FOR SUCCESSFUL PATIENT MANAGEMENT

- HCV RNA virus load is important throughout treatment with PIs to determine:

  -Eligibility for shortened therapy (RGT)

  -Discontinuation of therapy due to futility

  -Risk of developing resistance

  - Assessment of response at the end of treatment (EOT)

  -Confirmation of response 24 weeks after the end of treatment (SVR)

CHALLENGES WITH USING HCV RNA ASSAYS IN MONITORING RESPONSE

- Current HCV RNA assays used in practice have different quantifiable ranges (LLOD and LLOQ)

- Package inserts for licensed PIs specify different time points for monitoring HCV RNA as well as different HCV RNA thresholds used for defining treatment futility

CHALLENGES WITH USING HCV RNA ASSAYS IN MONITORING RESPONSE

- In-house and commercially available quantitative HCV RNA assays have differing LLOD and LLOQ
- LLOQ=Lowest HCV RNA concentration within linear range of assay, i.e. smallest amount of HCV RNA that can be detected and accurately quantified
- LLOD=Lowest amount of HCV RNA concentration that can be detected with 95% probability

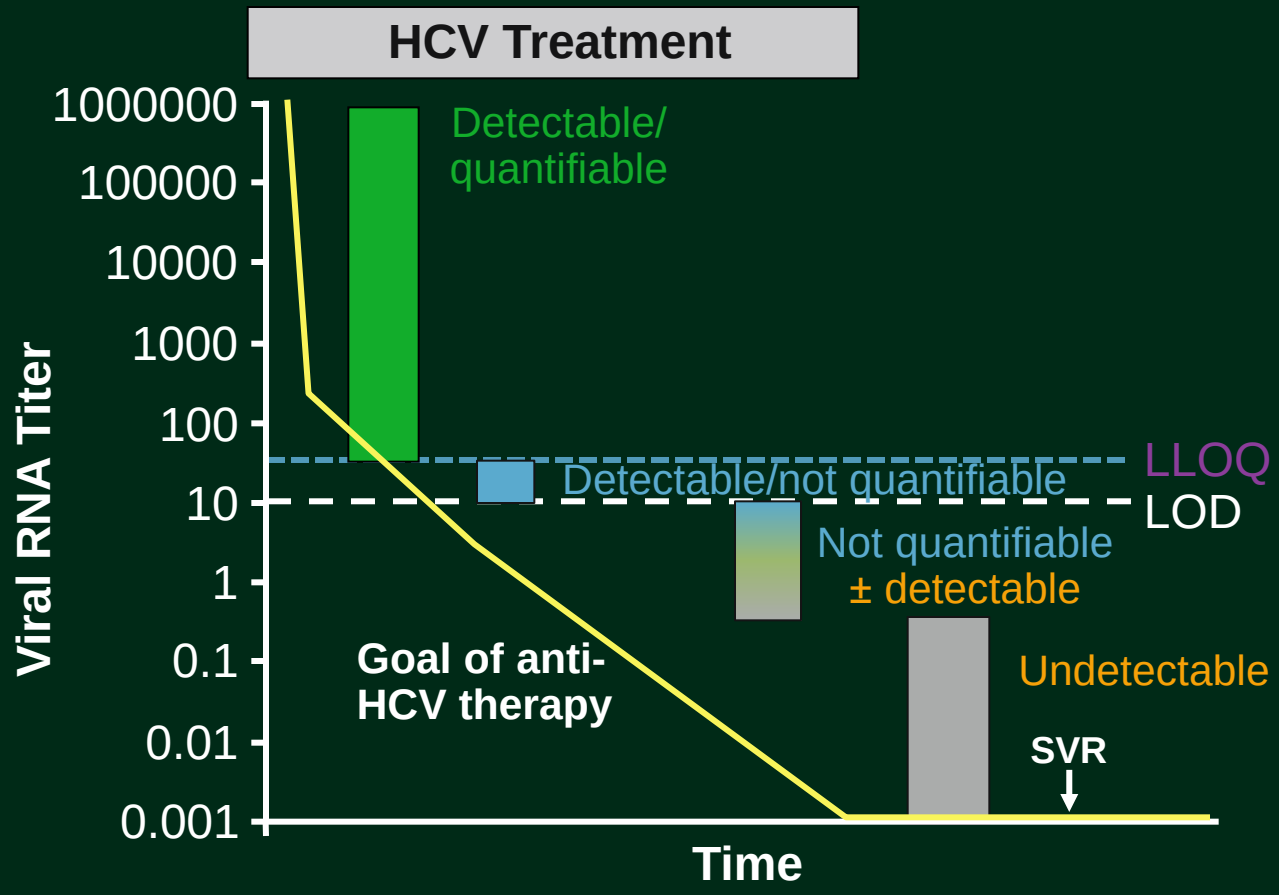
QUANTITATIVE HCV RNA ASSAYS IN USE IN THE UK

Assay	Method	Dynamic Range in IU/ml	LLOD	LLOQ = LLOD
Abbott m 2000	RT-PCR	12-100,000,000	12	Yes
COBAS TaqMan HCV Test v2.0	RT-PCR	25-300,000,000	15	No
QIAGEN artus HCV QS-RGQ	RT-PCR	35-177,000,000	21	No
In-house PCR	RT-PCR	50-1,000,000	25	No

RECOMMENDATIONS FOR USE OF HCV RNA ASSAYS IN PATIENTS ON PIs

- Can we really accurately detect LLOD of 10 IU/ml?
- Only 2 commercially available assays in UK fit recommendations
- Do we change technology to adhere to recommendations? (not const-effective solution)
- Do we buy time pending the release of new therapies?
- Do we ignore recommendations?

HCV RNA Levels and Relationship to LLOD and LLOQ



Adapted from Naeger LK, et al. Intl Workshop on Clinical Pharmacology of Hepatitis Therapy 2011. Abstract R-8.

NORFOLK & NORWICH UNIVERSITY HOSPITAL EXPERIENCE WITH PIs

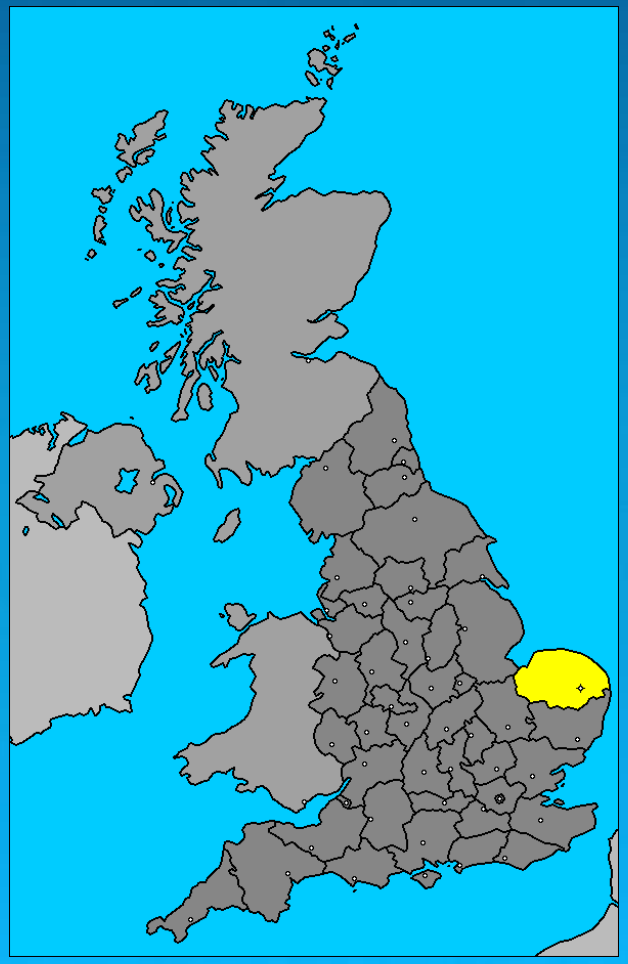
- East of England has 20 major hospitals serving 5.6 million people
- £8.1 billion on health care annually
- Laboratory confirmed HCV infection in East of England in 2012: 688 new cases

- Patient pool: IV drug use, blood transfusion in the past

- Prevalence of HCV in PWID 49%

- Four prisons in Norfolk

- Prison Services conduct screening for HCV infection
- Hepatitis nurses visit prisons



NNUH EXPERIENCE WITH PIs

- NNUH-University Hospital, 1000 beds
- Catchment area: 1,500,000 people
- Located between the Biomedical Research Centre with 3,500 scientists and the University of East Anglia
- New building to accommodate scientists working on translational research
- NNUH new hub for NIHR Clinical Research Network for the whole of East of England

NNUH EXPERIENCE WITH PIs

- Hub for the Eastern Pathology Alliance
- Virology and Bacteriology Services for 3 Trusts and GPs in Norfolk
- Specialist Virology Centre with expertise in hepatotropic viruses
- 350,000 samples processed annually
- 3 Consultants Virologists, 3 Specialty Trainees in Virology
- 40 BMSs and 2 Clinical Scientists
- Multidisciplinary Team dealing with patients on triple therapy

PIs TREATMENT MONITORING AT NNUH

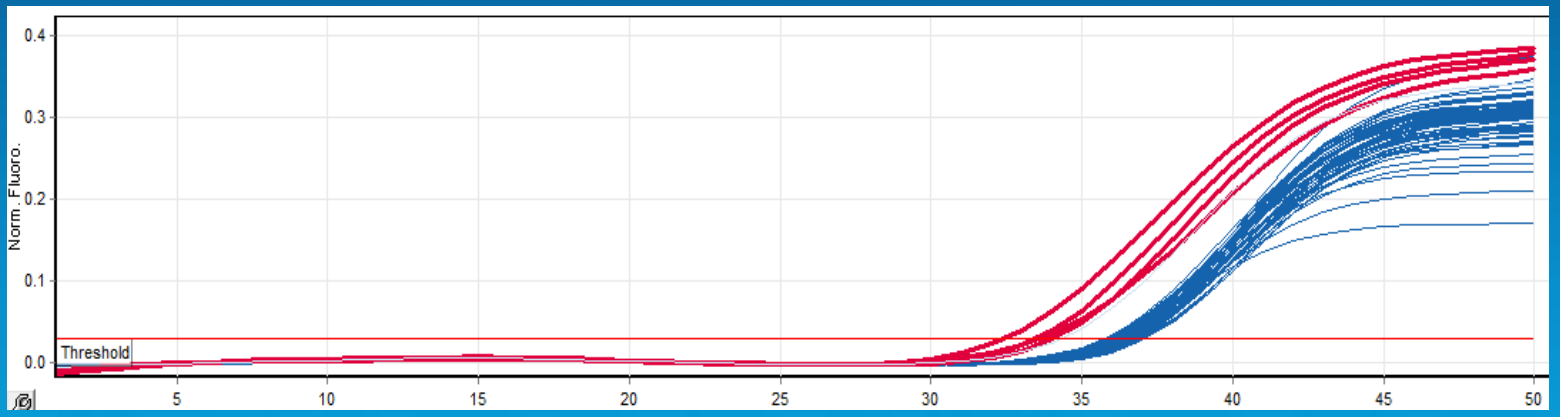
- Assay used Qiagen artus HCV QS-RGQ
- Method: RT-PCR
- Dynamic range: 35-177,000,000 IU/ml
- LLOD: 21 IU/ml (previously 36 IU/ml)
- Sample type: EDTA blood, (extraction vol 1 ml, elution vol 60 µl)
- Run frequency: 2 weekly
- Monitoring response to triple therapy: results within 12 hours

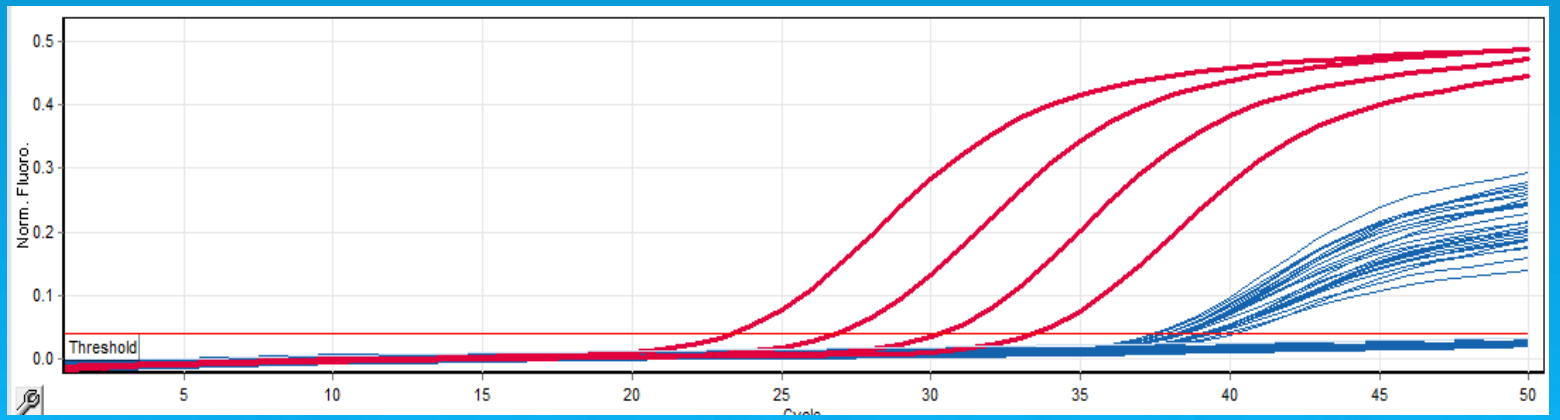
INVESTIGATION OF QS-RGQ DETECTION LIMIT FOR HCV AT NNUH

- WHO 4th International Standard 06/102
- Diluent: Basematrix Diluent B001-008 (Seracare Lifesciences)
- HCV QS-RGQ kit (72) version 1
- RNA extraction using the QIAsymphony DSP Virus/Pathogen kit
- From the stock at 260,000 IU/ml dilutions were made to 20, 15 and 10 IU/ml
- 12 replicates (1.2ml) of each dilutions were made

INVESTIGATION OF QS-RGQ DETECTION LIMIT FOR HCV AT NNUH

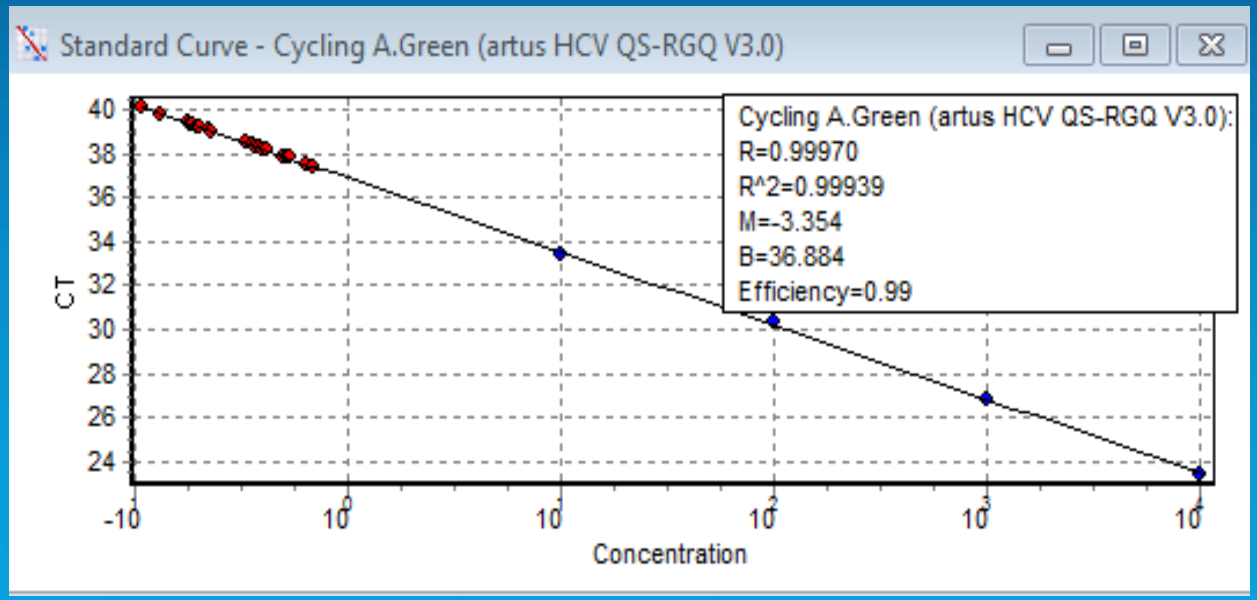
Orange (IC) channel)





Green (HCV) channel

INVESTIGATION OF QS-RGQ DETECTION LIMIT FOR HCV AT NNUH



Hit rates:

10 IU/ml	4/12	33%
15 IU/ml	5/12	42%
20 IU/ml	9/12	75%
26 IU/ml	9/12	75%

Artus® HCV QS-RGQ Kit

Performance characteristics-Qiagen, January 2013

(Page 2, artus HCV QS-RGQ Ki, Version 1, REF 4518363,4518366)

Hit rate analysis for HCV LLOD study

HCV titre (IU/ml)	Total replicate number	Total Number Positive	Percentage of positives
Probit analysis **30**	**32**	**32**	**100**
20	**60**	**59**	**98**
15	**60**	**51**	**85**
5	**60**	**40**	**67**

PIs TREATMENT MONITORING AT NNUH

- Triple therapy for genotype 1 patients started from 1st week of January 2013

Total Genotype 1	Genotype 1, subtype a	Genotype 1, subtype b	Male	Female	Telaprevir	Boceprevir
27	21	6	20	7	24	3
100%	78%	22%	74%	26%	89%	11.1%

22 patients >4 weeks, 5 <4 weeks on therapy

PIs TREATMENT MONITORING AT NNUH

Results at 4 weeks (Telaprevir therapy)

- 20 patients on Telaprevir >4 weeks on therapy
- 15 (75%): HCV target not detected
- 2 (10%): HCV detected <21 IU/ml (below LLOD)
- 2 (10%): HCV detected (<1,000 IU/ml)
- 1 (5%): HCV detected (>100,000 IU/ml)

PIs TREATMENT MONITORING AT NNUH

- 75% of patients on TVR eligible for shorter (24 weeks) therapy
- 20% of patients on TVR will have 48 weeks of therapy
- 5% of patients on TVR will discontinue treatment due to lack of response

CONCLUSIONS

- Qiagen artus HCV QS-RGQ RT-PCR performs well in monitoring response to triple therapy with Pis
- Analysis of the response to treatment in larger patient cohort will give a clearer picture
- New potent classes of drugs will further improve response to therapy making recommendations regarding LLOD and LLOQ less relEvant

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THANK YOU
FOR YOUR ATTENTION

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