BACKGROUND: The current treatment options for hepatitis C virus (HCV), based on direct acting antivirals (DAA), are dependent on virus genotype and previous treatment experience. Treatment failures have been associated with detection of resistance-associated substitutions (RASs) in the DAA targets of HCV, the NS3, NS5A and NS5B proteins. OBJECTIVE: To develop a next generation sequencing based method that provides genotype and detection of HCV NS3, NS5A, and NS5B RASs without prior knowledge of sample genotype. STUDY DESIGN: In total, 101 residual plasma samples from patients with HCV covering 10 different viral subtypes across 4 genotypes with viral loads of 3.84-7.61 Log IU/mL were included. All samples were de-identified and consequently prior treatment status for patients was unknown. Almost full open reading frame amplicons (∼9kb) were generated using RT-PCR with a single primer set. The resulting amplicons were sequenced with high throughput sequencing and analysed using an in-house developed script for detecting RASs. RESULTS: The method successfully amplified and sequenced 94% (95/101) of samples with an average coverage of 14,035; four of six failed samples were genotype 4a. Samples analysed twice yielded reproducible nucleotide frequencies across all sites. RASs were detected in 21/95 (22%) samples at a 15% threshold. The method identified one patient infected with two genotype 2b variants, and the presence of subgenomic deletion variants in 8 (8.4%) of 95 successfully sequenced samples. CONCLUSIONS: The presented method may provide identification of HCV genotype, RASs detection, and detect multiple HCV infection without prior knowledge of sample genotype.