How is the serological diagnosis of hepatitis B and C in Brazil?

Folgueras-Flatschart, A.V. 1, Pessoa, M. C. F. 2, Flatschart, R.B. 3

1 Researcher - Labio/Dimav, INMETRO – Duque de Caxias-RJ, Brazil
2 Researcher - Dicla/Cgere, INMETRO – Duque de Caxias-RJ, Brazil
3 Researcher - Lamac/Dimav, INMETRO – Duque de Caxias-RJ, Brazil

rbflatschart@inmetro.gov.br

Abstract. Hepatitis B and C are a serious public health problem that can be chronic and lead to cirrhosis or cancer. Their accurate diagnosis are important to allow proper treatment. In this work we seek, through analysis of data from Proficiency Testing rounds, to understand the performance of laboratories their serological tests. Considering the final interpretation of quantitative and qualitative assays, we observed at least 95% agreement on the results obtained in relation to those expected by the provider. This rate was higher in antibody detection assays, with a slight tendency to increased agreement and decreased dispersion over the period.

1. Introduction
Clinical Laboratories provides a fundamental service in support of diagnosis, treatment and monitoring of diseases, so quality assurance of assays is a requirement to make this tasks effective. Clinical laboratory activities are highly regulated and the risk of operating without complying with minimum legal requirements may result in fines, negative impact on the institution's image, and personal liability of the laboratory's Technical Responsible [1]. On Anvisa side, laboratories must comply, among other regulations, with RDC No. 050/02, RDC No. 302/05, RDC No. 306/04 and RDC No. 063/11. RDC 302 [2] establishes as a requirement that all clinical laboratories must participate in Proficiency Tests (PT) for all assays performed in their routine. Thus, laboratories should periodically participate in test rounds in which they perform analysis of unknown materials that simulate patient samples, with values known only by the provider. This practice is essential to highlight the quality of their analytical procedures (equipment operation, reagent quality, technical operator skill, QC material use, etc.) [1]. In Brazil only a few institutions provide these services, namely: the “Testing Proficiency” program of CONTROL LAB- Quality Control for Laboratories (linked to the Brazilian Society of Clinical Pathology / Laboratory Medicine - SBPC / ML) and the National Program for Quality Control (PNCQ) (linked to SBAC - Brazilian Society of Clinical Analysis).

The National Institute of Metrology, Quality and Technology (Inmetro), a fundamental institution in promoting the harmonization of consumer relations, innovation and competitiveness in the country, has as its main tasks the production of Reference Materials (RM). Centralizing the actions of biometry, the Directorate of Metrology Applied to Life Sciences (Dimav) has sought to know the market for RM applicable to tasks related to laboratory diagnosis of diseases caused by viruses. These items should be used as quality control materials for the evaluation of reagents, kits or assay methods or even in the production of SI-traceable secondary standards. In a previous initiative, Inmetro (in partnership with
SBPC / ML, ANVISA and MS) conducted a consultation with private clinical laboratories and public laboratories from the States to map out for which viruses the development of reference materials would be of greater interest. At the time we confirmed the initial perception on the importance of MR for viruses commonly tested for blood donation: HIV, HTLV, and Hepatitis B and C [3]. Now in this work we seek, through the analysis of data from Proficiency Tests promoted between 2012 and 2015 by Control Lab (linked to SBPC / ML), to understand the performance of laboratories and to know the market of in vitro diagnosis tests by serology for Hepatitis B and C.

### 1.1. Hepatitis

As in the rest of the world, viral hepatitis in Brazil is a notifiable disease and a serious public health concern. From 1999 to 2017, 718,837 cases were reported, with 40,198 new cases of viral hepatitis in 2017, according to the Ministry of Health (MS) Secretariat for Health Surveillance (SVS) [4]. The most common viral hepatitis is type A, B and C, but the latter two (caused by the HBV and HCV viruses) are considered more serious because viral infection can become chronic, leading to severe liver problems such as cirrhosis or cancer, events of high morbidity and mortality.

Early and accurate diagnosis of these infections allows for the appropriate treatment of the disease and has a direct impact on the individual's quality of life, defining the moment of onset, the type and effectiveness of treatment and even the screening for hepatocellular carcinoma (HCC) when occurs.

#### 1.1.1. Hepatitis B

Viral Hepatitis B is caused by the HBV virus (belonging to the family Hepadnaviridae) and is usually oligosymptomatic (causing few or no characteristic symptoms). In adult individuals exposed exclusively to HBV, spontaneous cure occurs in about 90% of cases, but the disease can progress into chronic form in approximately 5% to 10% of these. In 20% to 25% of chronic cases, liver disease progresses to an advanced condition that can lead to cirrhosis or cancer [5].

To monitor HBV infection, serum immunity markers (anti-HBs), HBV surface antigen (HBsAg) and bloodstream virus quantification (viral load / HBV-DNA) are used. The appearance of antibodies against the virus surface antigen (Anti-HBs) and the disappearance of HBsAg and viral load, for example, indicate resolution of HBV infection in most cases. In the evolution to chronic infection, in turn, the virus persists (with the presence of HBsAg) for more than six months, detected by serological tests [5]. In the identification of potentially infected individuals, detection of antibodies against the viral core (Anti-HBc) allows the differentiation of cases of natural infection from those of vaccine protection, where only antibodies against the surface antigen will be present (Anti-HBs) [6].

#### 1.1.2. Hepatitis C

Viral Hepatitis C is caused by the HCV virus (belonging to the Flaviviridae family), which is also asymptomatic in most cases. However, it has a much higher rate of evolution to chronic disease, reaching 80% of those infected. About 20% of those chronically infected with HCV can progress to liver cirrhosis and about 1% to 5% to liver cancer. The prevalence of reacting people (antiHCV) is estimated to be approximately 0.7%, which corresponds to about 1,032,000 HCV reactive people in Brazil [7]. Among viral hepatitis, Hepatitis C continues to be the target of the highest number of reported cases: 11.9 cases per 100,000 inhabitants [4].

Anti-HCV is the marker that indicates prior contact with the virus. Thus, to confirm the infection, the initial test is performed by screening these antibodies by classical serological methodology or rapid tests. If this first test is reagent, an additional molecular biology test to confirm viral replication should be done [7].

### 2. Methodology

Data on the rounds of the “Proficiency Test” program were obtained from Control Lab - Quality Control for Laboratories, linked to the Brazilian Society of Clinical Pathology / Laboratory Medicine (SBPC / ML). The results of “Serology II” PT rounds were evaluated, covering Quantitative, Qualitative or Confirmatory serological tests for the detection of Hepatitis B virus antigen and antibodies (HBsAg, Anti-HBs and Anti-HBsA tests respectively). HBc) and for the detection of antibodies against Hepatitis
C virus (Anti-HCV tests). In each round, 2 reactive items and 2 nonreactive items (without this identification) were sent to each participant. The period evaluated covered 11 rounds (from December 2012 to December 2015), with a total of 11,164 participations. Then, in more detail, the 8 quarterly rounds that took place between March 2014 and December 2015 were analyzed. The files shared by the provider was in PDF format. The Excel program was used for tabulation and analysis of the results of the EP rounds.

In analyzes, the number of participants, the agreement of the interpretation of the results in relation to the expected results and the methodologies used were considered. A picture of the kit market has also been analyzed, considering global and national manufacturers of in vitro diagnostic products for this area.

3. Results and Discussion
Eleven rounds (between December 2012 and December 2015) of “Serology II” Proficiency Tests organized by Control Lab were evaluated for the detection of antigen and antibodies against Hepatitis B virus (HBsAg, Anti-HBs and Anti-HBc) and detection of antibodies against Hepatitis C virus (AntiHCV) (Table 1).

Table 1 - Number of participations in the rounds of the “Serology II” Proficiency Test program organized by Control Lab for quantitative (Quant), qualitative (Quali) and confirmatory (Confirm) tests for hepatitis B virus antigen and antibodies (respectively HBsAg, Anti-HBs and Anti-HBc) and for antibodies against Hepatitis C virus (Anti-HCV).

| Assays            | dic_2012 | jun_2013 | dic_2014 | mar_2014 | jun_2014 | sep_2014 | dic_2015 | mar_2015 | jun_2015 | sep_2015 | dic_2015 |
|-------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| HbsAg (Quant & Qual) | 264      | 274      | 266      | 266      | 282      | 278      | 273      | 285      | 291      | 285      | 287      |
| HbsAg (Confirm)   | 14       | 12       | 14       | 13       | 16       | 19       | 9        | 11       | 11       | 12       | 14       |
| Anti HBc (Quant & Qual) | 213  | 215      | 205      | 210      | 223      | 220      | 213      | 217      | 218      | 216      | 220      |
| Anti HBs (Quant & Qual) | 232  | 236      | 223      | 226      | 237      | 240      | 237      | 238      | 243      | 242      | 244      |
| Anti HCV (Quant & Qual) | 251  | 256      | 251      | 252      | 266      | 275      | 262      | 273      | 281      | 282      | 282      |
| Anti HCV (Confirm) | 8        | 6        | 5        | 6        | 7        | 8        | 5        | 6        | 6        | 6        | 6        |
| Total in each round at Serology II (HBV &HCV) | 982 | 999      | 964      | 973      | 1,031    | 1,040    | 999      | 1,030    | 1,050    | 1,043    | 1,053    |

More than 11,000 participants were registered in the period and there was a constant adherence of laboratories to the program, with a slight upward trend mainly in the HBsAg (for Hepatitis B) and AntiHCV (for Hepatitis C) assays (Figure 1). The offer of PT service and the constant work of the provider to convince laboratories is important for the adherence to the rounds, especially considering that, besides the obligation, there is the involvement of costs for the participants.
Analyzing all the data, considering the final interpretation of the result for the quantitative and qualitative tests, we observed that the agreement of the results obtained by all participants in relation to the results expected by the Control Lab provider was greater than 95%, except for the Anti-HCV test occurred in the Jun/2013 round (Figure 2). Furthermore, we observed that the percentage of agreement of the antibody assays (Anti-HBs, Anti-HBc and Anti-HCV) (Figure 2 - b, c and d) was, in general, higher than the observed for the HBsAg assay (Figure 2 - a), with a tendency for this percentage of agreement to increase and the dispersion to decrease over time. Thus, we understand that participation in the “Proficiency Test” program has been important for the continuous improvement of the quality of the results obtained in the routine of the participating laboratories.

For Hepatitis B (HBsAg) and Hepatitis C (Anti-HCV) detection assays, samples were also sent for confirmatory tests. In these cases, the agreement of the results of all participants was, for some rounds, equal to 100%, but in others this rate was only 80% (Figure 3).

Considering that much of the quality of laboratory assay results depends not only on the performance of the performing team, but also on the technique used, the quality of the reagents and controls, and the maintenance and calibration of equipment, we turn our attention to manufacturers of in vitro diagnostic kits and equipments during the 2014 and 2015 rounds (Table 2). Knowing these suppliers can also point us to potential customers for future Reference Materials developed for the area.
Figure 2 - Percentage of agreement of the results obtained by all participants in relation to the results expected by the Control Lab provider (from December 2012 to December 2015). The final interpretation of the result was considered for quantitative (Quant) and qualitative (Qual) tests for: a) Hepatitis B virus surface antigen (HBsAg), b) antibodies against Hepatitis B virus surface antigen (Anti-HBs), c) antibodies against the core of the Hepatitis B virus (Anti-HBc) and d) antibodies against the Hepatitis C virus (Anti-HCV).

Figure 3 - Percentage of agreement of the results obtained by all participants in relation to the results expected by the Control Lab provider (from December 2012 to December 2015). The final results of confirmatory tests were considered for: a) Hepatitis B virus surface antigen (HBsAg) and b) antibodies against Hepatitis C virus (Anti-HCV).
Table 2 - Participation in the serological diagnosis market for Hepatitis B and C, according to data from the 2014 and 2015 rounds of the “Proficiency Test” - “Serology II” program, organized by Control Lab for quantitative, qualitative and confirmatory antigen testing, antibodies against Hepatitis B virus (HBsAg, Anti-HBs and Anti-HBc respectively) and antibodies against Hepatitis C virus (Anti-HCV).

### HBsAg, Anti-HBc, Anti-HBs, Anti-HCV (Quantitative Systems)

#### 7752 participants

| Company                  | Origin        | Participants | %  |
|--------------------------|---------------|--------------|----|
| Roche Diagnostics        | Switzerland   | 2211         | 28,52|
| Abbott                   | USA           | 2191         | 28,26|
| Siemens Healthineers     | Germany       | 1088         | 14,04|
| Ortho-Clinical Diagnostics | USA       | 614          | 7,92 |
| DiaSorin                 | Italy         | 523          | 6,75 |
| Wama Diagnostica         | Brazil (São Carlos-SP) | 513 | 6,62 |
| Biomerieux Diagnostics   | France        | 256          | 3,30 |
| Access Diagnostics       | United Kingdom| 120          | 1,55 |
| Symbiosis Diagnostica    | Brazil (Leme-SP) | 86   | 1,11 |
| Biokit                   | Spain         | 44           | 0,57 |
| Autobio                  | China         | 42           | 0,54 |
| Bioclin Quibasa          | Brazil (Belo Horizonte – MG) | 18 | 0,23 |
| Diagnostics Bioprobes    | Italy         | 15           | 0,19 |
| In Vitro Diagnóstica    | Brazil (Belo Horizonte – MG) | 14 | 0,18 |
| Wiener Lab               | Argentina     | 14           | 0,18 |
| Bio-Rad                  | USA           | 3            | 0,04 |

### HBsAg, Anti-HBs, Anti-HCV (Qualitative Systems)

#### 745 participants

| Company                  | Origin        | Participants | %  |
|--------------------------|---------------|--------------|----|
| Wama Diagnostica         | Brazil (São Carlos-SP) | 362 | 48,59|
| Biomerieux Diagnostics   | France        | 173          | 23,22|
| Orange Life              | Brazil (Rio de Janeiro – RJ) | 114 | 15,30|
| Standard Diagnostic Inc  | Republic of Corea | 45   | 6,04 |
| Gold Analisa Diagnóstico | Brazil (Belo Horizonte – MG) | 22 | 2,95 |
| Human                   | Germany       | 14           | 1,88 |
| Doles                   | Brazil (Goiânia – GO) | 12   | 1,61 |
| Bioclin/Quibasa         | Brazil (Belo Horizonte – MG) | 3   | 0,40 |

### HBsAg (Confirmatory tests)

#### 81 participants

| Company                  | Origin        | Participants | %  |
|--------------------------|---------------|--------------|----|
| Abbott                   | USA           | 40           | 49,38|
| Roche Diagnostics        | Switzerland   | 38           | 46,91|
| Biomerieux Diagnostics   | France        | 3            | 3,70 |

### Anti-HCV (Confirmatory tests)

#### 30 participants

| Company                  | Origin        | Participants | %  |
|--------------------------|---------------|--------------|----|
| Bio-Rad                  | USA           | 16           | 53,33|
| Innogenetics NV          | Belgium       | 8            | 26,67|
| Mikrogen Disgnostik      | Germany       | 4            | 13,33|
| Biokit                   | Spain         | 1            | 3,33 |
| MP Biomedicals Suisse    | Switzerland   | 1            | 3,33 |

#### HBsAg, Anti-HBc, Anti-HBs, Anti-HCV (Quantitative Systems)

#### 7752 participants

### HBsAg, Anti-HBc, Anti-HBs, Anti-HCV (Qualitative Systems)

#### 745 participants

### HBsAg (Confirmatory tests)

#### 81 participants

### Anti-HCV (Confirmatory tests)

#### 30 participants
As expected and described earlier [1], we note the strong presence of international companies in this market. However, we were surprised by the important presence of Brazilian companies in the qualitative assay market for the detection of these viruses. Particular attention was drawn to the fact that these companies are headquartered in cities with strong links to public universities with tradition in scientific research in the areas of Biological / Biomedical Sciences and Biotechnology and well-known technology hubs that support innovative projects in their insertion in the market such as the São Carlos Technology Park (São Carlos-SP), the UFRJ Biotechnology Center (Rio de Janeiro-RJ), the Samambaia Technology Park (Goiânia-GO) and the Biominas (Belo Horizonte-MG). Only one of the companies, based in LemeSP, has a different profile.

The development of diagnostic kits and reagents in the area of Clinical Analysis is a process that requires highly qualified people, rigorous method validation steps and strict quality control. Constant customer contact for training, troubleshooting and problem solving is also essential. Thus, this is not a simple market, especially considering its direct relationship with the population's health. Innovating in this area is hard and complex, but economically it may be worth considering the values that the market moves. The world market for in vitro diagnostics should already surpass the $ 50 billion dollars mark, with the serological testing sector accounting for $ 15.8 billion dollars in business [8]. According to 2017 data from the Brazilian Chamber of Laboratory Diagnostics [9], in 2016, the in vitro diagnostic market in Brazil moved over R$ 2.8 billion (US 710 million), with a 10.5% increase over the 2015 result.

Brazil has been a pioneer in the world in many public health actions, including the universal treatment of AIDS patients, the rapid identification of the Zika epidemic associated with microcephaly cases and, recently, the announcement of the National Plan for the Elimination of Hepatitis C until 2030. This goal was presented during the opening of the “Cúpula Mundial de Hepatites 2017 -World Hepatitis Summit” – at São Paulo-SP (Brazil). According to the minister of health, we are one of the first countries to put into practice the proposal that is still under discussion in the world. To achieve this goal, we should increase testing and diagnosis of the disease throughout the population [10]. In the worldwide actions to eradicate hepatitis C, new national and international markets should be opened for proven diagnostic products. This is an important opportunity for the country to support its established (or even new) companies in the development, validation, registration, commercialization and internationalization of qualitative and rapid tests.

4. Conclusions
The analysis of the final data of the Proficiency Testing rounds of serological tests for Hepatitis B and C, allowed us to know the general performance of the laboratories and the Brazilian market of kits, reagents and equipment in this area.

Considering the number of participations from December 2012 to December 2015, we observed a constant adherence of laboratories to the program, with a slight upward trend. This is evidence of the provider’s constant work of convincing and offering the PT service to the labs. This dedication is important because adherence to the rounds, even compulsory, involves costs for participants.

Considering the final interpretation of the quantitative and qualitative HBsAg, Anti-HBs, Anti-HBe and Anti-HBC assay results, we found that the agreement of the results obtained by all participants with the results expected by the Control Lab provider was greater than 95%. There was a single point exception in 2013. Moreover, the percentage of agreement of the antibody tests (Anti-HBs, Anti-HBe and Anti-HCV) was generally higher than that found in the HBsAg antigen assay, with a tendency for increased agreement and decreased dispersion over the period. Thus, we understand that participation in the Proficiency Test program has been important for improving the quality of the results obtained in the routine of the participating laboratories.

Focusing on the analytical systems (kits, reagents and methods) used by the participants of the 8 rounds from March 2014 to December 2015, we observe the strong presence of international companies in this market, as expected. However, we noticed the important presence of Brazilian companies in the qualitative testing market for the detection of these viruses. Just as there are initiatives, such as SEBRAE’s [1], to support Clinical Laboratories, we believe that government support actions should be
intensified to national companies operating in the area of in vitro diagnostics, highlighting the validation processes, registration and marketing in international markets. These industries, mainly offering qualitative and rapid tests, would thus have access to markets created by global epidemic monitoring and disease eradication campaigns.

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