

Contextual analysis of the first SARS-CoV-2 RNA screening period in nasopharyngeal swabs, 2020-2022: a comparison of two diagnostic tests

Analisi del contesto prima epoca screening per SARS-CoV-2 RNA in tamponi rinofaringei, 2020-2022: comparazione tra due metodologie

Elisabetta Scomparin¹, Roberta Di Matteo², Valentina Bigucci¹, Valentina Repetto¹, Haymanot Bonelli¹, Erika Berta¹, Tatiana Bolgeo², Antonio Maconi³

¹SC Microbiology and Virology, Azienda Ospedaliero-Universitaria SS Antonio e Biagio e Cesare Arrigo, Alessandria; ²SC Research Training Innovation Infrastructure, Department of Research and Innovation, Azienda Ospedaliero-Universitaria SS Antonio e Biagio e Cesare Arrigo, Alessandria; ³Director, Research and Innovation Department (DAIRI), Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Italy

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ABSTRACT

Background: exceptionally consistent COVID-19 laboratory diagnostics are crucial for case identification, patient management and contact tracing. The Coronavirus-2019 (COVID-19) pandemic affected over 771.407.825 people up until October 2023, with over 6.9 million deaths. The current process of clinical laboratory consolidation, impacting large geographic areas, presents an opportunity for the efficient and cost-effective implementation of novel laboratory technologies, as well as advancements in translational research and development. The aim of this study was to assess which of the two instruments could offer the most effective support to our laboratory's activities, minimizing errors during the pre-analytical phase, optimizing human resources, and reducing the Turn-Around-Time (TAT).

Materials and Methods: the diagnostic instruments available in the Microbiology laboratory of the Azienda Ospedaliero-Universitaria SS Antonio e Biagio e Cesare Arrigo (AOUAL) were the COBAS 6800 and the ALINITY platforms.

Conclusions: the Alinity platform offers clinicians a more user-friendly approach to understanding patient infectivity, compared to the closed Cobas system. It permits clinicians to review curves and access a cumulative Cycle Threshold (Ct), facilitating the hypothesis of acute or initial/final infection stages. This positions Alinity by Abbott as the preferred system over other instruments.

Background: una diagnostica di laboratorio COVID-19 eccezionalmente affidabile è essenziale per l'identificazione dei casi, la gestione dei pazienti e la ricerca dei contatti. La pandemia di Coronavirus-2019 (COVID-19) ha colpito oltre 135 milioni di persone al 10 aprile 2021, con oltre 2,9 milioni di decessi. Ha colpito 771.407.825 milioni di persone al 18 ottobre 2023, con oltre 6,9 milioni di morti. L'attuale processo di consolidamento dei laboratori clinici, che ha un impatto su vaste aree geografiche, offre la possibilità di introdurre in modo efficiente ed economico nuove tecnologie di laboratorio e di promuovere la ricerca e lo sviluppo traslazionale.

Materiali e Metodi: gli strumenti analitici a disposizione del laboratorio di Microbiologia dell'Azienda Ospedaliero-Universitaria SS Antonio e Cesare Arrigo di Alessandria (AOUAL) erano gli analizzatori COBAS 6800 e ALINITY.

Conclusioni: l'analizzatore Alinity offre ai medici un approccio più accessibile alla comprensione dell'infettività del paziente, a differenza del sistema chiuso Cobas. Consente ai clinici di visualizzare le curve e di accedere a un CT cumulativo (ciclo soglia), permettendo di ipotizzare l'infezione acuta o iniziale/finale. Ciò rende Alinity m Abbott un sistema preferibile ad altri.

Introduction

Exceptionally reliable COVID-19 laboratory diagnostics are crucial for case identification, patient management, and contact tracing. As of April 10, 2021, the Coronavirus-2019 (COVID-19) pandemic had affected over 135 million people, with over 2.9 million deaths. By October 18, 2023, the pandemic had impacted over 771,407,825 individuals, resulting in over 6.9 million deaths.

In 2020, following the first wave outbreak of COVID-19, the

“Cure Italy” decree (Decree Law No. 18/2020, converted into Law No. 27/2020) authorized an increase in healthcare funding for the year 2020, amounting to 1,410 million euros. This investment was allocated for staff recruitment, recognition of salary increases related to the special working conditions during the emergency phase, procurement of equipments and services, as well as the purchase of services to be supplied by accredited private facilities if necessary. It also facilitated the establishment of Special Units of Continuity of Care (USCA).

Despite the government's initial investment, clinical laboratories are facing direct challenges due to healthcare issues such as increasing consolidation, rising prices, reductions in reimbursement, and staff shortages. Molecular laboratories are striving to increase operational productivity to establish themselves as reliable healthcare partners. However, factors such as population density, dynamics, and composition, disease prevalence and severity, economic status, and the cost of tests exert significant pressure on these infrastructures.

Additional challenges stem from shifts in healthcare delivery, government and payer initiatives, the stance of insurance organizations, consumer education and expectations, and rapid technological advancements.¹

Considering these aspects, the current process of clinical laboratory consolidation, which impacts large geographic areas, provides an opportunity for the efficient and economical introduction of novel laboratory technologies, as well as the advancement of translational research and development.¹

These various stressors have resulted in a shift toward consolidating biomedical laboratory testing, where resources and services are centralized and serve a larger population, promoting greater efficiency, standardization, and potentially faster time-to-results. Initially motivated primarily by commercial concerns, such as diagnostic costs, privatization and shortage of specifically qualified personnel, consolidation efforts have evolved to offer additional benefits. These include the integration of databases and reporting systems, as well as more easily managed biorepositories. Moreover, emerging technologies and platforms can be seamlessly integrated into larger laboratories. These up-to-date and often automated technologies used in established laboratories already require (multiple) levels of accreditation to comply with Conformité Européenne (CE) or US Food and Drug Administration (FDA) guidelines.¹

Furthermore, regulatory validations are becoming increasingly important to address the production of innovative diagnostics in response to the rising rates of healthcare-associated infections and Antimicrobial Resistance (AMR).

Reorganisation of the AOUAL Microbiology Laboratory

Between February and March 2020, following the first wave of the COVID-19 pandemic, the SC Microbiology laboratory at the Azienda Ospedaliero-Universitaria SS Antonio e Biagio e Cesare Arrigo (AOUAL) underwent significant reorganization.

The laboratory commenced COVID-19 emergency operations on March 6, 2020. Initially, it processed only 72 samples per day, with 6 runs of 12 samples, employing a completely manual RNA extraction and subsequent amplification approach.

As of 17 March 2020, another production line was introduced, capable of analyzing 12 samples per instrument at a time using a semi-automated method, totaling 36 samples per run every three hours. However, the laboratory was already equipped with an analytical platform previously used for other tests. At the end of March, the analysis capacity increased to about 300 samples per day, which remained insufficient given the high daily swab intake.

At the beginning of April, Roche distributed Covid-19 molecular biology research reagents, consistent with the Cobas 6800 automated instrument (Roche, Basel, Swiss) at AOUAL, with a capacity of 700 swabs per day.

Handling this extensive swab analysis, along with complementary activities, like receiving, sorting, labelling and secondary tube

sampling, was all performed manually by the microbiology laboratory technicians and administrative staff. These staff members were also responsible for entering results into the Laboratory Information System (LIS) and simultaneously onto the regional platform. The volume of swabs was so substantial that it was necessary to use the Cobas 6800 analyzer, located and catalogued outside the Microbiology Laboratory, but within the AOUAL structure, in the hematological ward.

During this period, work organization became more challenging, with staff transporting swabs from the microbiology lab to the laboratory technicians in the hematological ward, who then loaded the sessions onto the Cobas 6800 analyzer. Due to the workload, the analysis was coded into the management system, and the flow acceptance and reports were computerized and shared with ASL AL and ASL AT. There were discussions about increasing swab numbers and intensifying contact tracing, often without considering the necessary resources in terms of equipment and manpower.

The main challenges faced by the AOUAL Microbiology Laboratory were the lack of instrumentation and reagents. Specific equipment required for swab analysis is not universally available in all analytical laboratories, and each analysis must be conducted under maximum safety conditions. The global demand for analysis led to a shortage of reagents.

In response to the new production requirements imposed by COVID-19 and new technological implementations, it was decided, in agreement with AOUAL's management, to re-evaluate the laboratories capacity. the AOUAL Microbiology Laboratory is part of the regional reference laboratory designated by the Piedmont Region.

Following the Circular No. 9774 of March 20, 2020, which mandated validated equipment and trained/qualified staff to execute protocols for viral genome extraction and RT-qPCR tests procedures that are identical and standard for all regions, in alignment with the National Reference Laboratory of the Istituto Superiore di Sanità (ISS) and adhering to the most recent procedures. Furthermore, biological samples designated for diagnosing SARS-CoV-2 had to be managed in compliance with the World Health Organization's (WHO) biocontainment regulations to prevent any risk of contamination to staff and the environment.

Considering the nationwide spread of SARS-CoV-2 and the pandemic status officially declared by the WHO on March 11, 2020, in line with guidance from the European Centre for Disease Prevention and Control (ECDC) and subsequent WHO's recommendations on laboratory diagnosis, it was decided that regional reference laboratories should act as coordinating centers for additional laboratories identified by the regions to conduct SARS-CoV-2 diagnosis. These regional reference laboratories were tasked with providing the necessary support and guidance according to specific regional plans.

In response to the number of swabs performed in the Piedmont region and the productivity required by the Inter-Agency Department for Infectious Diseases and Emergencies (DIRMEI), Abbott's ALINITY (Abbott laboratories, USA) emerged as the optimal choice meeting the company's needs. This instrument allows continuous sample loading, overcoming batch logic. Subsequently, AOUAL developed an action plan to meet a hypothetical demand of >1000 swabs per day, seven days a week.

The aim of this study was to evaluate which of the two instruments could offer the most suitable support to the laboratory's activities, reducing errors during the pre-analytical phase, optimizing human resources, and minimizing the Turn-Around-Time.

Materials and Methods

The COVID-19 swab samples processed by the AOUAL Microbiology Laboratory, serving as a regional reference laboratory, included samples from emergency and reception facilities, in addition to all inpatient facilities within both the Adult and Children's AOUAL Hospital Presidiums, as well as the Hospital's "Teresio Borsalino" rehabilitation facility. Moreover, all facilities that were affiliated with the local territorial health service of Alessandria (ASL AL) and the local territorial health service of Asti (ASL AT), including territorial hotspots and the carers of the infected population. Furthermore, during the first pandemic period, swabs from Aosta, Cuneo and Turin were also processed. The analytical instruments available to the laboratory were the COBAS 6800 and the ALINITY platforms.

COBAS 6800 System

In the microbiology laboratory, the COBAS 6800 system enables nucleic acid analysis based on Polymerase Chain Reaction (PCR) through an automated and integrated workflow. Both PCR set-up and real-time PCR are fully automated processes, including the extraction of total nucleic acids directly from primary and secondary tubes. From sample processing to result interpretation, the instrument streamlines instrument use, consumables, reagents, and data management in a single efficient workflow.

The Cobas SARS-CoV-2 Test is a two-target, real-time Reverse Transcriptase PCR (RT-PCR) assay for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swab specimens.

One target is the viral ORF1, a region exclusive to SARS-CoV-2 (target 1), and the second is a conserved region in the E gene for pan-Sarbecovirus detection (target 2). The test employs an internal RNA control for sample preparation and PCR amplification control, and the uracil N-glycosylase system to prevent PCR contamination.

The manufacturer's software assigns test results, providing automated data management. Results can be viewed directly on the system screen, printed, or transferred to a Laboratory Information System (LIS). According to the manufacturer's instructions, a tested specimen was considered positive for SARS-CoV-2 if Cobas detected positive results for both the ORF1 (target 1) and E (target 2) genes or for the ORF1 (target 1) gene only. If only the E (target 2) gene was positive, the result was reported as presumptive positive for SARS-CoV-2. Testing was conducted in batches of 94 samples plus one negative and one positive control.²

The Cobas® 6800 System allows up to 864 determinations in an eight-hour shift and up to 1,440 results in 24 hours. Achieving up to 8 hours of walk-away time with only 3 user interactions per run; it also optimizes resource-intensive applications such as viral load monitoring.³

ALINITY System

The Alinity m system is a fully integrated and automated molecular diagnostic analyzer using real-time PCR technology. It provides random access, continuous loading of samples, reagents, consumables, and access to waste, ensuring a fast time to first result and high throughput.

For routine PCR testing, four to six platforms are typically required, leading to inefficiencies, and requiring more staff training and expertise. Alinity m, however, offers true random access without compromising turnaround time or throughput. It features a universal sample rack – eliminating the need for sample sorting and simplifying the front-end workflow. The system delivers first results in less than 150 minutes and accommodates up to 20 assays on-board simultaneously.⁴

The Alinity m SARS-CoV-2 assay employs real-time RT-PCR technology for the qualitative detection of nucleic acid from SARS-CoV-2. It can analyze mid-turbinate nasal, anterior nasal, Nasopharyngeal (NP), and Oropharyngeal (OP) swabs, as well as Bronchoalveolar Lavage (BAL) specimens. These samples are collected from individuals suspected of having COVID-19 by their Healthcare Provider (HCP), including those without symptoms or other reasons to suspect COVID-19.

The Alinity m SARS-CoV-2 assay is a dual target assay for the RdRp and N genes. To ensure correct sample preparation, an unrelated RNA sequence, distinct from the SARS-CoV-2 sequence, is introduced into each specimen at the beginning of the process. This unrelated RNA sequence is concurrently amplified by RT-PCR, serving as an Internal Control (IC) to validate the accurate execution of the procedure for each sample.⁵

Results

The data presented in the table illustrate the enhanced efficiency of laboratory technicians' work through the utilization of primary tubes. This improvement is evident in terms of biosafety, time saved during the loading of biological samples into the instrument, and the resultant reduction in Turnaround Time (TAT).

The decrease in errors during the preanalytical phase has positively impacted the overall output, thereby facilitating the clinical management of patients. The ability to observe Cycle Threshold (Ct) values has promoted more effective communication with clinicians. Specifically, a Ct value greater than or equal to 35 has signaled the end of isolation and enabled the transfer of patients to other departments. This, in turn, has freed up beds for more critical patients, optimizing bed management.

Despite encountering challenges such as demanding maintenance and occasional standstills, the numerous advantages outlined in the table were decisive factors in selecting the Alinity m for our workflow.

Table 1 shows the differences between the two analysis systems.

Number of swabs processed - duration of tests - for how long they were used in hospital

Between March 2020 and October 2020, 44,465 the COBAS 6800 system processed 44,465 molecular swabs. In response to the hospital's needs, AOUAL introduced Abbott's ALINITY instrument.

Since its implementation in October 2021, the ALINITY analyzer has processed 23,554 molecular swabs until the end of 2022. This system enables the continuous loading of samples, overcoming batch logic.

Year 2021: 71,200

Year (May) 2022: 12,872

Table 1. Differences between the Cobas 6800 and the Alinity system.

Cobas	Alinity
Closed System	Open System
Loading with 96 samples every 1.5 hours, with results every 3 hours	Non-stop loading, random access, results every 2.5 hours
Secondary tube processing: high possibility of pre-analytical error due to the large number of samples	Mother-tube processing leading to a potential zero pre-analytical error
Increased biological risk of environmental contamination due to the transfer of samples	Lower biological risk of environmental contamination due to sample transfer
Positive and negative control per analytical session, in case of control failure the entire analytical session must be repeated	Positive and negative control every 48 hours in relation to the reagent batch. Each individual sample has an internal control: if it is not amplified, only that sample is aborted and repeated
No possibility of checking internal amplification curves	Possibility of controlling the PCR curve
It is not possible to load a single sample in an emergency	Possibility of loading emergencies
2 CTs, one for each Target gene	1 single Ct stemming from the sum of the Cts from the two Target genes
Easy maintenance	High maintenance
Small amount of waste	Substantial waste from bulky plastic supplies
Less internal contamination	Increased internal contamination
Frequent machine standstills	Frequent machine standstills
Presence of dUTP/UNG chemical amplicon contamination prevention system	Absence of dUTP/UNG chemical amplicon contamination prevention system
Reagent costs covered by our facility	Reagent costs reduced thanks to regional distribution at the expense of the Civil Protection Department

Discussion

The Roche Cobas system proved to be an asset for the AOUAL Microbiology Laboratory during the initial phase of the pandemic: i) it demonstrated a very high performance in terms of result accuracy and sample processing capacity; ii) the undeniable advantage of having a sister instrument located at the AOUAL blood transfusion centre, serving as either a complement to the Microbiology Laboratory instrument or as a backup during downtimes.

While the Roche Cobas system was excellent, it had its drawbacks: i) the need for a secondary tube, involving the sampling of the Lan mother tube and re-labelling, increasing the risk in the pre-analytical phase; ii) limited visibility of the entire Ct, crucial for clinicians in the second phase of the pandemic; iii) ‘closed’ system processing only 94 samples per session, lacking the flexibility to address urgent samples frequently encountered at AOUAL.

The introduction of Abbott’s Alinity m analyser undoubtedly brought about significant changes within the laboratory: i) improved TAT from sample arrival in the laboratory to findings; ii) reduced man-machine time and reduced pre-analytical errors; iii) random access loading and an urgent sample processing mode enabled the management of patient emergencies requiring immediate hospitalization or urgent surgery that could not be postponed - in practical terms, the ‘urgent channel’ specimen processing option permitted specimens to be held while urgently essential swabs were prioritized; iv) processing the main tube with barcode labelling (nasopharyngeal swab), streamlined the technical operator’s task and optimized resources at a time when healthcare staff are in short supply; v) near-zero intra-laboratory pre-analytic errors with the adoption of the primary test tube.

Drawbacks included: i) high maintenance; ii) substantial waste from bulky plastic supplies; iii) increased internal contamination; iv) frequent machine standstills; v) absence of dUTP/UNG chemical amplicon contamination prevention system.

Conclusions

In conclusion, within our context, Alinity significantly improved the organization of the Covid-19 diagnostic routine, reducing pre-analytical errors, TAT, and operator time activity. The Alinity analyser provided clinicians with essential insights, allowing them to view curves and access cumulative CT, supporting a better understanding of patient infectivity. The choice of Alinity m Abbott over other systems was based on these advantages.

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Correspondence: Roberta Di Matteo, SC Research Training Innovation Infrastructure, Department of Research and Innovation, Azienda Ospedaliero-Universitaria SS Antonio e Biagio e Cesare Arrigo, via Venezia 16, 15121 Alessandria, Italy.
Tel. 0131-206309. E-mail: rdimatteo@ospedale.al.it

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