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Contribution of Automated Antigen Tests, the LumiraDx Ag Test in the Response during the Second Wave of the COVID-19 Pandemic in Bangui

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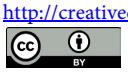
Abstract

Context and objective: The COVID-19 pandemic has become a major public health problem and has mobilized many innovative means of diagnosis. The Central African Republic is not spared. The emergence of variants and their impact require health monitoring despite the obligation of vaccination. The purpose of this campaign was to determine the circulation of pending second-wave variants. **Patients and Methods:** A second mass screening campaign took place from 02 to 22 July 2021 in the main land and river entry points of Bangui (Exit North-PK12, Exit South-PK9, Port Beach) and at the LNBCSP. Antigenic and RT-PCR tests carried out on nasopharyngeal samples made it possible to select strains which were finally sequenced. **Results:** Of 2687 participants included in the study, 53 (1.97%) were positive for SARS-CoV-2. Thirteen (1.53%) were male and 40 (2.18%) female. The analyses carried out on the LumiraDx analyzer were positive for 109 samples against 53 on the



RT-PCR. The prevalence was higher in the most tested age groups (30 to 50 years) with two clusters identified. B.1.617.2 (Delta) variants were predominant (57%). **Conclusion:** SARS-CoV-2 continues to circulate. The acquisition of automated antigenic tests (LumiraDx®) with sensitivity and specificity close to those of the reference test (RT-PCR) will allow better mass diagnosis for an optimization of the surveillance of COVID-19 in our countries with limited resources. The predominance of the B.1.617.2 (Delta) variant would suggest a third wave in the Central African Republic.

Keywords

COVID-19, Automated Antigen Testing

1. Introduction

COVID-19, with its 767 million confirmed cases and 6.9 million deaths recorded until July 2, 2023 [1], is one of the most devastating pandemics recorded since the last century, after the Spanish flu. Declared a pandemic on 11 March 2020 by the WHO Director-General, who recommended mass laboratory testing as the main backbone of the response, significant efforts and resources have been deployed, but not commensurate with the threat [2] [3] [4].

As of 28 January 2021, the World Health Organization noted that the number of COVID-19 cases and deaths is rising sharply in Africa as new, more contagious variants of the virus spread to other countries [5].

More than 175,000 new COVID-19 cases and more than 6200 deaths were recorded in Africa during the week of 18 - 24 January 2021, while the number of infections increased by 50% on the continent between 29 December 2020 and 25 January 2021, compared to the previous four weeks. In week 3, South Africa saw a slight decrease in cases, but 22 countries continue to see a sharp increase in cases. The number of deaths doubled over the same four-week period, with more than 15,000 deaths concentrated mainly in 10 nations in southern and northern Africa [6] [7].

The 501Y variant, V2, initially identified in South Africa, is predominant and fueling a record number of cases in South Africa and the sub-region. This variant has been found in Botswana, Ghana, Kenya, the French Indian Ocean region of Mayotte, Zambia and 24 non-African countries [7] [8] [9] [10] [11].

The variant initially detected in the UK was found in Gambia and Nigeria. More research was therefore needed to determine whether the new strain causes more severe disease [8] [10].

Faced with new variants detected in the United Kingdom, Brazil and South Africa that had multiple mutations in genes encoding spike (S) proteins, authorities have taken drastic measures and warned against the false negativity of some RT-PCR tests whose primers target the S gene, while antigen tests, most of which target the N protein, are mostly spared [4] [7] [12] [13] [14] [15] [16].

The Central African Republic, after the detection of its first case on March 14, 2020, had then experienced two successive waves, the most recent of which were devastating due to the circulation of variants B.1.1.7 (commonly known as the British variant) and B.1.135 (South African variant) highlighted thanks to the multilateral efforts deployed during the mass campaign launched on March 17, 2021 and coordinated by the technical team of the National Laboratory of Clinical Biology and Health Public [17]. This activity was decisive in the response since it not only made it possible to identify clusters in community settings, but also made it possible to obtain relevant data that served as a compass for the vaccination campaign that began on March 19, 2021 [18].

Given the risk of new waves already deplored by African countries such as the DRC and Nigeria, a complementary mass campaign that was part of the post-vaccination surveillance and response, was strongly recommended [11] [19] [20].

The National Laboratory, which had experience and well-established human resources in these types of campaigns, had recently been equipped with LumiraDx® semi-automatic analyzers. The baseline assessments and performance of these automated antigen testing methods justify the use of these new tools for a decisive new campaign in the national response. Because in the Central African Republic, no other automated antigen test for the detection of SARS-CoV-2 was available before this campaign. Antigenic tests, although recognized as having low sensitivity compared to RT-PCR, have the advantage of speed of rendering results and ease of use in a context of low availability of RT-PCR tests. In addition, the use of automated platforms makes it possible to remedy the sensitivity problems of immunochromatographic tests [3] [4].

It is in this context that this study was conducted with the aim of mass screening by automated antigen tests and identifying clusters and variants of interest of SARS-CoV-2.

2. Patients and Methods

2.1. Study Framework

The National Laboratory of Clinical Biology and Public Health is chosen to coordinate the recruitment within it and the technique of the samples collected in the 04 other sensitive sites chosen that have been:

- The northern entrance to PK26 Route de Boali (C.A.R);
- The South entrance PK 9 Route de M'Baïki (C.A.R);
- Port Beach giving access to the ZONGO gateway southern border with the Democratic Republic of Congo (D.R.C).

2.2. Type of Study

This was a descriptive prevalence study based on mass screening campaign data.

2.3. Sampling

It is made up of consenting people screened by antigenic tests and RT-PCR tests

during this study.

2.3.1. Inclusion Criteria

Included in this study were volunteers over 10 years of age who wanted to know their SARS-CoV-2 infectious status who consented to nasopharyngeal swabs (PN).

2.3.2. Non-Inclusion Criteria

Refusal to participate or last-minute withdrawal were the grounds for non-inclusion.

2.4. Conduct of the Survey and Data Collection

This study was conducted by a multidisciplinary team from the National Laboratory of Clinical Biology and Public Health under the coordination of the National Committee for the Response to COVID-19.

After a brief sensitization based on the previous one in relation to the first mass campaign in March 2021, volunteers were either directed to the LNBCSP or directed to the identified sites for their sampling and antigen tests, their results were returned on average after about 30 minutes to 2 hours depending on the workload of the site technicians. The report cards of the results and the recruitment form (see annexes) were the main tools for managing information relating not only to surveillance but also to the care of patients who will in the meantime be referred to the National COVID-19 Care Center or to health facilities identified according to the proximity of their residences to the sites.

Probabilistic estimates based on the 2% prevalence and known sensitivity and specifics of the test compared to RT-PCR allowed us to predict 31 positive and 1969 negative samples, at least 25 of which could meet the eligibility criteria for sequencing in this study, which included:

- SARS-CoV-2 infection at the beginning of vaccination;
- A discrepancy between NAAT and antigen tests that are positive while not detected by RT-PCR;
- Virulent or devastating forms of COVID-19;
- Symptomatic confirmed by both with a low viral load.

2.5. Procedures for Transporting Samples to the Laboratory and Techniques

Most samples will be collected at the LNBCSP in facilities prepared for this purpose given the requirement of the manufacturers. Those collected remotely from the site were promptly routed by the mobile team for testing within an hour of collection to minimize the risk of false-negative tests.

The LumiraDx SARS-CoV-2antigen test (LumiraDx UK Ltd., Dumyat Business Park, Alloa, FK10 2PB, UK) was used in the field testing patients according to the manufacturer's procedures. For this semi-automated immunofluoresence test using the principle of microfluidic detection, 400 microliters of samples were recovered in suspension in virological transport media (MTV) were deposited in

the then samples of the strip before being analyzed after about fifteen minutes. The remaining suspension was then transported to the LNBCSP where RT-PCR was performed on the ABI 7500 and CFX96, following the instructions of the manufacturers of the kits used.

RNA extracts or positive samples were sent in triple packaging to the INRB of KINSHASA via the UNHAS humanitarian airlift that regularly deserts the two capitals (Bangui and Kinshasa).

Figure 1 shows the flowchart summarizing the different key steps of the patient recruitment processes and the realization of their tests during this campaign.

2.6. Ethical Considerations

We received ethical clearance from the Ethics and Scientific Committee of the Faculty of Health Sciences (N32/FACSS/CES.2020).

3. Results

Out of 2682 samples analyzed, 109 were positive in the LumiraDx ® Antigen test. PCR confirmed 53 of these positive samples, giving 100% sensitivity and 97.9% specificity for LumiraDx compared to RT-PCR which is the gold standard diagnostic technique for COVID-19 (Table 1).

The average age of volunteers was 38 ± 12.20 years with extremes ranging from 1 to 75 years (Table 2).

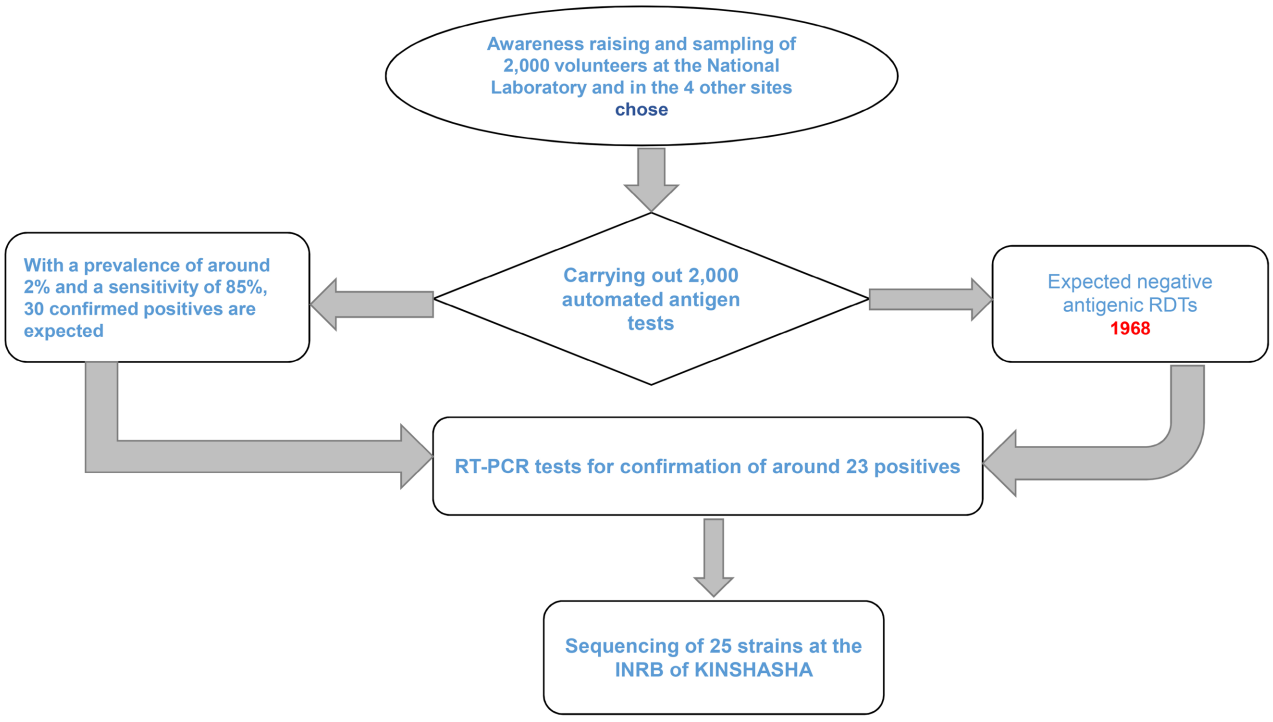


Figure 1. Flowchart of predefined activities before the mass COVID-19 testing campaign. Taking into account the prevalence and epidemiological trends, we planned to test 2000 patients and send 25 strains to be sequenced at the National Biomedical Research Institute of KINSHASA in the Democratic Republic of Congo.

Table 1. Results of evaluation of antigenic tests on LumiraDx® Antigen in comparison with RT-PCR tests carried out at the National Laboratory of Clinical Biology and Public Health.

Test results	RT-PCR SARS-CoV-2 (n = 2682)	
	Positifs	Négatifs
LumiraDx® tests	53	56
	0	2578
Total	53	2634

P-value = 0.001 ≤ 0.005. Se = VP/(VP + FN), so the probability of detecting the infection actually present in patients with COVID-19 is 100%. Sp = VN/(VN + FP), therefore the probability of screening people who are truly unaffected negative is 97.9%.

Table 2. Demographic and epidemiological characteristics of the study population using the LumiraDx® Antigen antigen test carried out at the National Laboratory.

Gender	LumiraDx ®tests		Total
	Positive	Negative	
F, n (%)	26 (3.07)	822 (96.93)	848
M, n (%)	83 (4.51)	1756 (95.49)	1839
Total	**109 (4.06)**	**2578 (95.94)**	**2687**
Sex-ratio H/F	3.19	2.14	2,17
Middle age	42.30	37.52	37,71
median	43.00	37.00	
Age class			
<1 year	0 (00.00)	1 (100.00)	1
1 - 10 years, n (%)	4 (7.84)	47 (92.16)	51
11 - 20 years, n (%)	4 (2.67)	146 (97.33)	150
21 - 30 years, n (%)	15 (2.35)	624 (97.65)	639
31 - 40 years, n (%)	21 (2.71)	754 (97.29)	775
41 - 50 years, n (%)	35 (5.57)	593 (94.43)	628
51 and more, n (%)	30 (6.77)	413 (93.23)	443
Total	**109** (4.06)	**2578** (95.94)	**2687**
Collection site			
NLCBPH[a]	88 (5.80)	1429 (94.20)	1517
PORT BEACH	16 (3.27)	474 (96.73)	490
PK[β]26	4 (0.95)	417 (99.05)	421
BIMBO	1 (0.39)	258 (97.33)	259
TOTAL	**109** (4.06)	**2578** (95.94)	**2687**

[a]**NLCBPH** or LNBCSP (in French): National Laboratory of Clinical Biology and Public Health. [β]**PK**: Mileage point.

The prevalence of SARS-CoV-2 infections was much higher in those under 10 years of age and in those over 40 years of age (Figure 2 and Figure 3).

The delta variant accounted for 57% of the 52 samples sequenced by Illumina® (Figure 4).

4. Discussion

Antigen tests, although recognized as much less sensitive than RT-PCR, offer practical solutions in terms of ease of use and speed of delivery of results during the pandemic at a time when RT-PCR tests were scarce on the market and waiting times for RT-PCR were abnormally long due to increased demand [3] [4] [21] [22] [23].

In our series, a sensitivity of 100% and a specificity of 97.6% were obtained with the LumiraDx ® Antigen test. Mbow in Senegal [21] on a panel of 184 samples report a sensitivity of 81.6% and a specificity of 100%, values slightly different from ours but also from those reported by the manufacturer (Sensitivity of 97.60% and Specificity of 96.60%).

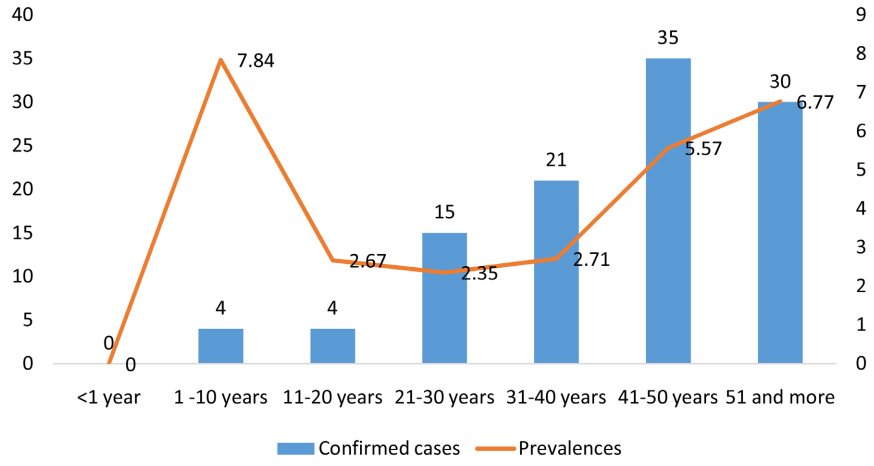


Figure 2. Distribution of cases by age groups according to tests carried out on LumiraDx®.

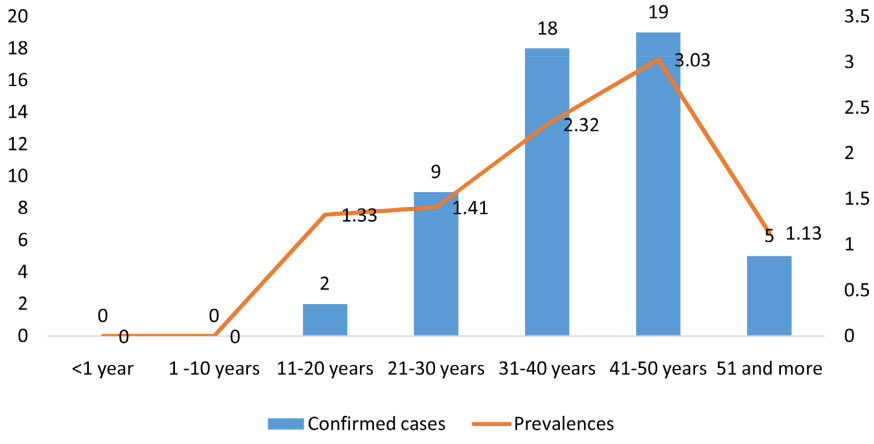


Figure 3. Distribution of cases by age groups according to the RT-PCR tests carried out.

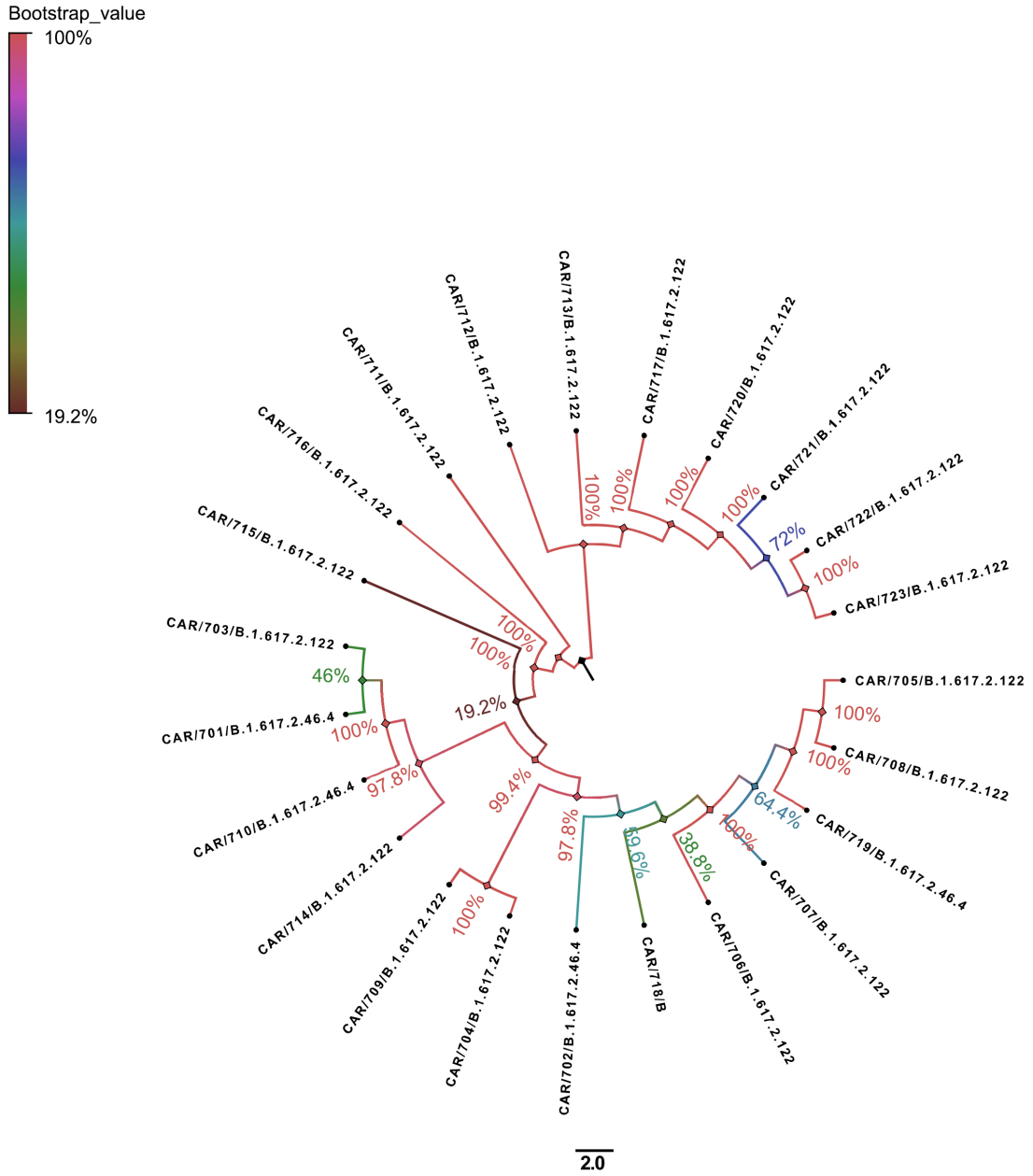


Figure 4. Phylogenetic tree of variants detected during this campaign. Sequence coverages generated are reported as percentages. We then noted a predominance of delta variants (57% of variants detected).

It should be remembered that the positivity of antigen tests is inversely proportional to the Cycle Threshold (Ct) values of positive samples [2] [21] [22]. Thus, in our series, 96% of samples positive for the LumiraDx® antigen test had a CT value below 30. Indeed, in the literature, antigenic tests are recognized as having lower sensitivity and specificity than RT-PCR [3] [4] [15]. But these poor performances can be improved thanks to automation technologies and with samples of low CT values (less than 30) [21].

This justifies these variations in performance depending on whether we are in an outbreak period where people infected en masse in a relatively short period of

time could have quite high viral loads and therefore enough viral particles to be detected by this antigen test [4] [23] [24] [25] [26].

The interest of this test was not only to help mass screening but also to solve possible problems related to certain RT-PCR tests using the Spike gene whose results could be difficult to interpret in the presence of new variants. Antigen tests could then detect these cases as long as the amount of viral load inversely proportional to the CT value would have allowed it [27] [28]. However, this campaign period corresponded to that of the circulation of the delta variant which was new and which had led to a very violent second wave in the Central African Republic in a context of low vaccination coverage.

The average age is 38 ± 12.20 years and those under 10 years of age were as affected as those over 40 years of age. It should be noted that at the beginning of the introduction of vaccination, the priority was the immunization of health care workers and people at risk. But as the additional doses were acquired by the health system, this campaign was extended to children and the rest of the population. The reluctance linked to the Post Injection Events (MAPI) had been a brake on the extension of vaccination coverage, even during the second wave.

5. Conclusion

This study carried out as part of a mass campaign using a new screening technology which has been successfully evaluated shows that it is important to increase screening capacities with automated antigen tests in countries with limited resources. However, the low sampling and the local unavailability of sequencing techniques are the obvious limitations. In the future, we recommend an extension of recruitment sites across the entire territory. The fairly recent implementation of genomic surveillance will boost the monitoring of variants and improve the response.

Authors' Contributions

We certify that all authors have contributed to the realization of the article whether in the technique, coordination and document review.

CDR, ELY, PS, SP, JEKK, EJK, RMS, BK: project design and laboratory techniques.

CDR, NK, LSH, OS, WSN, FXMK, LB, GG, JDDL, BK, AS, PS: data analysis and outcome interpretations and coordination of screening activities.

CDR, NK, LSH, WSN, FXMK, LB, GG, JDDL, BK, MMS, RDB, AS, CDMK, RNN, AS: Manuscript elaboration and document review.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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