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Evaluation of the artus® Prep&Amp UM RT-PCR for detection of SARS-CoV-2 from nasopharyngeal swabs without prior nucleic acid eluate extraction

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ABSTRACT

Here we describe a retrospective clinical evaluation of the QIAGEN artus® SARS-CoV-2 Prep&Amp UM RT-PCR assay that detects SARS-CoV-2 RNA without the need for a nucleic acid eluate extraction procedure. Using Roche SARS-CoV-2 RT-PCR on the cobas® 8800 platform as a reference standard, a total of 225 confirmed SARS-CoV-2 positive and 320 negative nasopharyngeal swabs in viral transport media, were used to evaluate the artus® assay. Using the RT-PCR cycle threshold as a semi-quantitative marker of viral load, an assessment of over 370,000 SARS-CoV-2 RT-PCR positive results was used in the design of the reference positive specimen cohort. The viral load of all reference positive specimens used in the evaluation was a unique and accurate representation of the range and levels of SARS-CoV-2 positivity observed over a 13-month period of the COVID-19 pandemic. The artus® RT-PCR detects the presence of SARS-CoV-2 RNA, an internal control, and the human RNase P gene to ensure specimen quality. The diagnostic sensitivity of artus® was 92.89% with a specificity of 100%. To assess the analytical sensitivity, a limit of detection was performed using the 14th WHO NIBSC SARS-CoV-2 international standard, recording a 95% LOD of 1.1×10⁵ IU/ml. The total invalid rate of specimens was 7.34% due to a lack of detectable RNase P (C >35). The artus® SARS-CoV-2 Prep&Amp UM RT-PCR assay is a new rapid RT-PCR assay, which may be considered to produce acceptable levels of diagnostic sensitivity and specificity whilst potentially halving the laboratory processing time.

1. Introduction

The speed and accuracy of diagnosis for any infectious pathogen is essential to limit the spread of infection and target appropriate treatment. Low assay sensitivity or delays with reporting test results can prevent timely isolation of infected individuals and reduce the effectiveness of contact tracing [1]. At the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, limitations with laboratory reagents and consumables were experienced globally [2,3], exposing how unprepared the world was to face this new global diagnostic challenge. Scientists and biotechnology companies responded en masse, first increasing their production of laboratory consumables, and later, developing new assays dedicated to the rapid detection of SARS-CoV-2.

New technologies to identify SARS-CoV-2 have had varied success with hundreds of in vitro diagnostics (IVD) receiving emergency use authorization by both the U.S. Food and Drug Administration [4] and the UK Medicines and Healthcare products Regulatory Agency (MHRA) [5]. Rapid antigen point-of-care tests (RAT) have been an important tool for the early detection of SARS-CoV-2, reporting high specificity but with varied interpretations of sensitivity (48.9-76.8%) [6-8]; a statistic heavily dependent on viral load, the test cohort, and the operative performing the test [8]. New rapid laboratory tests such as reverse-transcription

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loop-mediated isothermal amplification (RT-LAMP) assay (both Direct and extracted RNA formats) also received emergency use authorisation in the UK, although much wider reported interpretations of diagnostic sensitivity (34% to 99%) [9–15] raises questions regarding methods and/or interpretation of such studies. The use of spiked samples and duplicate/triplicate testing possibly accounting for some of the variability between reports [9–13,15].

The inconsistencies in published results highlight the importance of proper guidance when validating/assessing new molecular procedures for the detection of SARS-CoV-2 [16]. Although the reported sensitivities of some rapid detection methods may be lower than usually accepted for clinical diagnostic tests, the ability to self-test (RAT), the speed at which individual results can be produced and the ability to detect high viral loads provide the main justifications for their use. The introduction of guidelines [16] from the UK government regarding the reporting of studies designed to properly assess the clinical performance of SARS-CoV-2 detection tests is welcomed and necessary to inform the scientific community of what is expected and acceptable.

Here we establish the clinical performance of the artus® Prep&Amp UM (QIAGEN, Hilden, Germany) RT-PCR for the rapid detection of SARS-CoV-2, using an RT-PCR reference standard with the highest reported detection of SARS-CoV-2 available to the Manchester University NHS Foundation Trust (MFT) and a sample set highly representative of the range of viral loads observed during a significant proportion of the SARS-CoV-2 pandemic. We explore the hypothesis that acceptable levels of diagnostic sensitivity and specificity can still be achieved whilst bypassing the time consuming, reagent-dependent, and costly nucleic acid extraction and purification process.

2. Materials and methods
2.1. Observed range of SARS-CoV-2 positive C\textsubscript{T} values over a substantial proportion of the pandemic

To determine the most representative cohort of clinical specimens to assess artus® RT-PCR, all SARS-CoV-2 RT-PCR results, from multiple platforms, were collected from the MFT laboratory information management system between the 01/04/2020 and 30/04/2021. To ensure the most accurate assessment of artus®, the Roche SARS-CoV-2 RT-PCR on the cobas® platform (Roche, Basel, Switzerland) was used as the reference standard, as the platform was considered to be the most sensitive SARS-CoV-2 detection platform at the MFT [5]. Sample inactivation and cobas® RT-PCR was performed as previously described [11]. To enable an accurate reflection for the distribution of positive SARS-CoV-2 results, the C\textsubscript{T} values for the ORF1ab gene from first-time positive specimens were used to map the distribution of viral loads received during the aforementioned 13-month pandemic period.

The distribution of C\textsubscript{T} values was plotted for first-time positive patients. Positive results were grouped within a single numerical C\textsubscript{T} value and the number of specimens recorded within each C\textsubscript{T} value expressed as a percentage of the total first-time positive results. Specimens used to assess the clinical sensitivity of artus® were chosen with a viral load and range representative of the first-time positive distribution.

2.2. Clinical specimen cohort to determine the diagnostic sensitivity and specificity

A total of 545 clinical nasopharyngeal swabs (NPS) collected in Remel® VTM were tested using the artus®, comprising of 225 confirmed SARS-CoV-2 positive and 320 negative specimens. All specimens tested using the artus® were unlinked and anonymised prior to blind testing. Of 225 positive NPS specimens, 193 were stored at -80°C prior to testing and 32 stored at ambient temperature and tested within 72 h of receipt. Positive and negative frozen NPS were chosen randomly between March and April 2021 based on ORF1ab C\textsubscript{T} detection values. All negative specimens were stored at -80°C prior to testing with the artus® assay. As only a limited number of artus® tests were available to the MFT, specimen numbers were in concordance with the guidance supplied by the MHRA Target Product Profile (TPP) for point-of-care SARS-CoV-2 detection tests [17].

2.3. Artus® SARS-CoV-2 Prep&Amp UM PCR

To ensure that viable virus was inactivated prior to testing, 50μl of specimen was placed in a MicroAmp® Optical 96-Well Reaction Plate (Thermo Fisher) and held at 95°C for 1 min on an Applied Biosystems 9700 Thermal Cycler (Thermo Fisher) as per Public Health England (PHE) SARS-CoV-2 heat inactivation protocol [18]. Specimens were cooled to 4°C prior to testing. Inactivated specimens were processed as per manufacturer’s instructions for use (IFU) [19]. Both negative and positive (AMPLIRUN® - Vircell, Grenada, Spain) extraction controls were used for each run of the artus® protocol. Amplification was performed on an ABI 7500 Fast system (Thermo Fisher). Detection of SARS-CoV-2 RNA, an RNase P human sampling control (HSC) and the Internal Control was performed using the three dye layers FAM, VIC and Cy5, respectively. Results were analysed using ABI 7500 Fast system software (Thermo Fisher) and Ct values determined using a baseline-corrected normalised reporter (ΔRn) threshold of 0.15. The validity of test runs were determined as per Table 1 and result interpretation as per Table 2. Any reference positive specimens that produced a negative SARS-CoV-2 result using the artus® assay was tested using the CDC assay and repeat tested on the cobas® platform. Additionally, six specimens for each of the of Alpha, Beta, Gamma, Delta and Omicron SARS-CoV-2 variants were tested, all with previous Ct values between 24 and 30.

2.4. Multiplex RT-PCR using the centre for disease control and prevention (CDC) 2019-nCoV N1&N2 assay with Beta-2-Microglobulin (β2M) as endogenous control

As the cobas® SARS-CoV-2 RT-PCR platform does not include an endogenous control, SARS-CoV-2 negative specimens that recorded a HSC Ct result >35 with the artus® assay were tested using the CDC Real time SARS-CoV-2 N1&N2 RT-PCR assay [20]. Briefly, specimens were inactivated as previously described [11] and extraction (DSP Virus/pathogen mini-kit, QIAGEN, Hilden, Germany) performed on the QIAsymphony (QIAGEN) as per manufacturer’s instructions. RT-PCR reactions consisted of 1.5μl of both the 2019 nCoV N1 and N2 primer/probe mix

| Control type | Detection in FAM dye (SARS-CoV-2) | Detection in VIC dye (HSC) | Detection in Cy5 dye (IC) | Interpretation |
|--------------|----------------------------------|---------------------------|--------------------------|---------------|
| AMPLIRUN® SARS-CoV-2 whole genome RNA control | Ct ≤39.00 | Indifferent | Indifferent | Run validated |
| | Ct ≥39.00 or No Ct | Indifferent | Indifferent | Run invalided |
| Manufacturer Positive Control | Ct ≤39.00 | Indifferent | Indifferent | Run validated |
| | Ct ≥39.00 or No Ct | Indifferent | Indifferent | Run invalided |
| No template control and Negative extraction control | Ct ≥39.00 or No Ct | C\textsubscript{T} ≥35.00 or No Ct | Yes | Run validated |
| | Any other combination with amplification in FAM or VIC dye | Indifferent | | Run invalided |
were for replicates analysis. Limit cobas®SARS-CoV-2 2.6.

The result was determined using a dilution of the 1st WHO International Standard for SARS-CoV-2 (NIBSC 20/146) [21]. A doubling dilution series of NIBSC 20/146 was created from an initial concentration of log 7.7 IU/ml to log 1.99 IU/ml using VTM as diluent. A Limit of Detection (LOD) was performed using multiple replicates per analysis. The number of replicates used for the cobas® and the artus® were as follows: 2 at log7.7, 6 at log6.7, 6 at log5.7 and 20 replicates for all other dilutions. The LOD for the CDC assay was taken from a previous in-house study and followed the same dilution series but used 8 replicates per dilution rather than 20 from log4.7 to log1.99. The LOD for the artus® assay was performed twice, once at the beginning of the study and once at the end. Probit analysis was performed in SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) software to determine 95% LOD in IU/ml.

2.7. Analytical specificity

Table 3 lists the molecular controls used to assess the analytical specificity and variant detection of the artus® RT-PCR.

3. Results

More than half a million results were downloaded from the laboratory management system over the 13-month period of the pandemic. A total of 374,606 valid swab samples were tested using the cobas® SARS-CoV-2 assay, with 357,977 recording a negative result and 16,629 recording a valid positive result. It was calculated that 2654 specimens were repeat positives within 90 days, leaving a total of 13,975 first-time positives over the specified period. The cobas® SARS-CoV-2 assay positive (ORF1ab) CΔ threshold interpretation was used to assess artus® is displayed in Fig. 1.

3.2. Sensitivity and specificity of the artus® SARS-CoV-2 Prep&Ampl UM RT-PCR

Using artus®, 209 specimens were recorded as positive from a total of 225 cobas® SARS-CoV-2 positive specimens (Table 4). From the 16 reference positive specimens that failed to produce a positive result with the artus®, 14 (7.3%) specimens were previously stored at -80°C and two (6.3%) were stored at ambient temperature. Seven of the frozen artus® false negative specimens recorded an ORF1ab CΔ of >35, 5 between 34-35, 3 between 33-34 and one at 32.48 (Table 5). The two false negative results recorded from specimens stored at ambient temperature had ORF1ab CΔ values of 34.3 and 38. Each false negative result produced a β2M CΔ <40 using the CDC assay. artus® demonstrated a diagnostic sensitivity and specificity of 92.89% and 100%, respectively (Table 4).

Analysis 1: Adopting the criteria stipulated in the manufacturers IFU (Table 2), a total of 40 specimens were considered invalid with the artus®, amounting to a specimen quality failure rate of 7.34% for the entire cohort of specimens and a reference negative failure rate of 12.5% (Table 4). Importantly, within the cohort of reference positive specimens, no invalid results were recorded.
Fig. 1. Ct values for first-time positive patients using the Roche Cobas 8800 RT-PCR platform overlayed with a mirrored percentage distribution of specimen numbers tested using the artus® SARS-CoV-2 assay.

Table 4
Analysis 1: Diagnostic sensitivity and specificity of the artus® SARS-CoV-2 Prep&Amp UM RT-PCR compared to Orf1ab detection using the Roche Cobas platform; Number of invalid specimens recorded with the Human Sampling Control cut-off set at Ct 35.

| Artus® Positive | Orf1ab RT-PCR Positive | Orf1ab RT-PCR Negative |
|----------------|------------------------|------------------------|
| Artus® Negative | Value                  | 95% CI                 |
| Sensitivity     | 92.89%                 | 88.71% to 95.88%       |
| Specificity     | 100.00%                | 98.69% to 100.00%      |
| Invalids - artus® human sampling control >35 Ct | All specimens (n545) | From negatives only (n320) |
| Number of artus® invalid specimens with /2M Ct of >40 | 40 (7.34%) | 40 (12.5%) |
|                 | 17 (3.12%)            | 17 (5.31%)            |

1 Three of the randomly chosen Orf1ab reference positive specimens were considered as false positives and removed from the study (Orf1ab Ct 30.01, 33.04 and 36.0) after confirmed repeat negative results on the cobas® assay and the CDC assay. Substitute specimens within the same Ct range were included and the mirrored Ct distribution range was unaffected.

2 Six of the 17 specimens produced a HSC Ct value between 35 and 40 with the artus® assay. The remaining 23 invalid specimens all recorded a /2M Ct value of <40, with 15 recording a HSC between Ct 35 and 40.

Analysis 2: Table 6 displays the number and percentage of invalid specimens if a Ct cut-off employed for the artus® was equivalent to the RNAse P target within the CDC 2019 nCoV N1N2 assay.

3.3. Turnaround times and analytical sensitivity and specificity

Table 7 displays the results for the analytical LOD performed on all assays used in the study, along with the average turnaround time for each protocol. The turnaround time using the artus® protocol was substantially quicker requiring only 1:42:00 in comparison to the cobas® SARS-CoV-2 reference standard, timed at 3:18:00.

Combined use of the ORF1ab and E genes targets on the cobas® platform produced the lowest LOD. From the two LOD analyses of the artus®, an average 95% LOD of approximately 1100 IU/ml was observed.

Table 3 displays the results for the analytical specificity of the artus® RT-PCR.

4. Discussion

RT-PCR remains the gold standard for the detection of most viral pathogens but requires the purity of a nucleic acid eluate extraction procedure to produce reliable results. This evaluation describes a rapid direct RT-PCR that produced a high level of diagnostic sensitivity and specificity without a lengthy extraction procedure, which can often account for half the process time. Direct-PCR without eluate extraction is not a new technique and it has been shown to be used reliably for pathogens in pure culture [22]. However, due to the nature of clinical specimens often containing an abundance of human material/PCR inhibitors, it is rarely used as a diagnostic tool for molecular testing [23].
Table 5
Positive results using the artus® SARS-CoV-2 Prep&Amp UM RT-PCR compared to the reference standard. Specimen numbers categorised by Ct value and prior storage.

| Cobas® Orf1ab Ct Value | No. of Orf1ab Ct positive Frozen specimens tested | No. of specimens positive using the artus® SARS-CoV-2 Prep&Amp | No. of specimens positive using the artus® SARS-CoV-2 Prep&Amp | Total positive reference specimens tested | Total artus® positives |
|------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|----------------------------------------|------------------------|
| 16.77                  | 1                                               | 1                                              | 1                                              | 1                                      | 1                      |
| 18.19                  | 4                                               | 4                                              | 1                                              | 1                                      | 5                      |
| 19.20                  | 6                                               | 6                                              | 1                                              | 1                                      | 6                      |
| 20.21                  | 8                                               | 8                                              | 1                                              | 1                                      | 9                      |
| 21.22                  | 9                                               | 9                                              | 2                                              | 2                                      | 11                     |
| 22.23                  | 10                                              | 10                                             | 2                                              | 2                                      | 12                     |
| 23.24                  | 11                                              | 11                                             | 2                                              | 2                                      | 13                     |
| 24.25                  | 11                                              | 11                                             | 2                                              | 2                                      | 13                     |
| 25.26                  | 11                                              | 11                                             | 2                                              | 2                                      | 13                     |
| 26.27                  | 11                                              | 11                                             | 2                                              | 2                                      | 13                     |
| 27.28                  | 11                                              | 11                                             | 1                                              | 1                                      | 12                     |
| 28.29                  | 11                                              | 11                                             | 2                                              | 2                                      | 13                     |
| 29.30                  | 13                                              | 13                                             | 2                                              | 2                                      | 15                     |
| 30.31                  | 14                                              | 14                                             | 2                                              | 2                                      | 16                     |
| 31.32                  | 16                                              | 16                                             | 2                                              | 2                                      | 18                     |
| 32.33                  | 16                                              | 15                                             | 2                                              | 2                                      | 18                     |
| 33.34                  | 13                                              | 10                                             | 2                                              | 2                                      | 15                     |
| 34.35                  | 9                                               | 5                                              | 2                                              | 1                                      | 11                     |
| 35.36                  | 5                                               | 1                                              | 2                                              | 1                                      | 7                      |
| >37                    | 1                                               | 0                                              | 1                                              | 0                                      | 2                      |

1 32 ambient temperature positive specimens were independently chosen to equally cover the range of Ct values from 18 to 36.

Table 6
Analysis 2: Invalid specimen numbers for the artus® assay when using a validity Ct result of <40 for the Human sampling control assay, comparable with RNase P assay in the 2019 nCoV_N1 and N2 protocol.

| All specimens (n545) | From negatives only (n320) |
|----------------------|----------------------------|
| Invalids - artus® human sampling control <40 Ct | 19 (3.49%) | 19 (5.9%) |
| Number of artus® invalid specimens with β2M Ct of >40 | 17 (3.12%) | 17 (5.31%) |

1 Adopting the Ct <40 cut-off for the artus® HSC, an additional 21 specimens would be deemed valid from the 40 invalid specimens. Using this criterion, only 19 of the negative specimens would be considered invalid amounting to a total HSC failure of only 3.49%; the adjustment not affecting the overall specificity of the artus® assay.

Table 7
Analytical 95% limit of detection for all diagnostic assays used in the study, including average time taken to result for each protocol used.

| Assay | Target | 95% LOD IU/ml | 95% CI IU/ml | Time to result |
|-------|--------|---------------|--------------|----------------|
| Cobas® SARS-CoV-2 (Extraction on Cobas 8800 closed system) | Orf1ab | 367.7 | 208.7 - 1343.5 | 3:18 hours (n9) |
| | E gene | 212.0 | 142.0 - 518.7 |
| | Orf1ab and E gene | 149.1 | 88.9 - 595.5 |
| CDC 2019 nCoV_N1 and N2 with β2M (Extraction on QIAasympohy) | N1 and N2 | 677.8 | 381.4 - 4313.8 |
| | | | | 7:09 hours (n2) |
| artus® SARS-CoV-2 Prep&Amp UM RT-PCR LOD 1 | N1 and N2 | 995.4 | 657.3 - 2678.5 |
| LOD 2 | N1 and N2 | 1184.2 | 894.4 - 2310.2 |

1 The CDC 2019-nCoV N1 and N2 assay produced an LOD with much wider confidence limits due to the reduced number of replicates tested.
2 Average time taken to final result from receipt of 96 inactivated specimens.

Interestingly, the distribution of Ct values for the 13,975 SARS-CoV-2 positive patients was not a typical bell-curve distribution but instead a plateau in positive patient numbers between Cₜ 23 and 29 and a peak between Cₜ 31 and 33 was observed (Fig. 1). We can speculate that the reason for this may be due to the viral trajectory of SARS-CoV-2, typified by a characteristic rapid short-lived peak of viral replication (mean Cₜ 23-28 for mild to severe symptoms [24,25]) followed by a slow decrease in viral RNA levels over several days or weeks [24–29]. As the MFT performs testing for SARS-CoV-2 on hospitalised patients, new admissions, asymptomatic staff and community outbreaks, the range of viral loads and the stage at which infection is detected, will be broad. Therefore, it is hypothesised that a significant proportion of the first-time positive specimens in our study are likely to be sampled outside the relatively short-lived peak viral load. Furthermore, it is not possible to determine to what degree we are observing viral loads from two independent patient characteristics, symptomatic and asymptomatic. One study of 97 positive participants showed symptomatic patients had an increased peak viral load compared to asymptomatic patients (Ct 23.3 vs 30.7) [26]. Notwithstanding the reasoning behind the distribution, we believe the cohort of specimens used in this evaluation was highly representative of the viral load distribution that would be encountered in a real-world laboratory scenario, allowing a more accurate assessment of diagnostic sensitivity.
With regards to the desired and acceptable criteria stipulated by the MHRA in the TPP for Laboratory-Based SARS-CoV-2 Viral Detection test [30], the artus® assay performed well as a rapid SARS-CoV-2 detection assay, with an analytical sensitivity of 110 IU/ml, a diagnostic sensitivity of 93%, and a diagnostic specificity of 100%, whilst also providing a concurrent assessment of test and specimen quality. Clinically, the three molecular targets used in the artus® RT-PCR supply the desired set of results when assessing the quality of any diagnostic PCR result; the level of human nucleic acid allowing clinicians to better interpret specimen quality and consider whether retesting is an appropriate course of action [31,32]. However, not all rapid assays that detect SARS-CoV-2 provide this scope of results. Rapid tests such as the Optigene LAMP assay does not provide an internal control or an reverse transcriptase control [33], nor does it provide a endogenous control to assess sample quality [34,35], not dissimilar to the RAT or Roche reference assay used in this study [36].

At first glance, the number of invalid results produced by the artus® assay interpretation was concerning. The 7.34% of specimens deemed invalid by the artus® (analysis 1) may reflect the overall quality of NPS received by our laboratory; the invalid interpretation conceivably reducing the number of false negatives and demonstrating an improved level of accuracy rather than test failure. However, it may also be that a proportion of these invalid results are due to a number of technical reasons, such as a reduced nucleic acid purity with the artus® chemical extraction process, specimen freeze thaw, RNA degradation over time, or a possibility that the HSC C₃ assay cut-off for the artus® protocol is overly stringent.

Exploring the contention that the HSC C₃ is too stringent, analysis 2 (Table 6) used the C₃ cut off (<40) instructed within the CDC N1/N2 SARS-CoV-2 assay [20] for the same RNase P target. This adjustment greatly decreased the number of overall invalids to only 3.5%, comparable to the invalid number of specimens recorded using the J2M target (3.12%).

One of the main limitations of this study was the lack of a comparable reference test. As no molecular test run at the MFT has all three of the RT-PCR targets in the artus®, a combination of assays was required to assess molecular targets to ensure the most stringent and fair comparison. However, the use of two platforms led to inconsistencies between levels of C₃ cut-off when assessing specimen quality, a discrepancy, we believe, mitigated by the additional analysis made for the endogenous control.

With regards to the practicality of the assay, the Remel® VTM used in this study contains a protein element that can form a precipitate during the inactivation procedure (95°C for 1 min). The physical precipitate did not seem to directly affect the results of the assay but may become more relevant with the implementation of automated testing and the potential of pipette tip blockage. However, the formation of precipitate may be mitigated by a change in inactivation procedure or use of an alternative VTM.

Further studies are required to assess if the extraction procedure used in this assay can be applied to other molecular techniques. It is accepted that eluate extraction procedures will remain best practice but dependant on the objective, along with continuous improvements to the extraction chemistry, there may be a place for non-eluate extraction within the molecular laboratory.

Consent for publication
All authors gave their consent for publication.

Availability of data and materials
All the data for this study will be made available upon request to the corresponding author.

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Declaration of Competing Interest
None.

Supplementary materials
Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcivp.2022.100098.

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