

# Seroprevalence of Syphilis, Human Immunodeficiency Virus and its Co-infection in Patients Attending an ICTC at a Tertiary Care Hospital in Villupuram, Tamil Nadu, India

K KAVITHA<sup>1</sup>, R DEEPA<sup>2</sup>, P BALAPRIYA<sup>3</sup>

## ABSTRACT

**Introduction:** Human Immunodeficiency Virus (HIV) and syphilis co-infection is common and affects similar age groups. The presence of syphilis infection increases the chances of transmission of HIV. Syphilis has a negative impact on HIV infection, resulting in increasing viral loads and decreasing Cluster of Differentiation 4 (CD4) cell counts during syphilis infection.

**Aim:** To determine the seroprevalence of syphilis, quantify HIV-Syphilis co-infection in patients attending an Integrated Counseling and Testing Centre (ICTC) centre.

**Materials and Methods:** This cross-sectional study was conducted at the Department of Microbiology, Government Villupuram Medical College, Tamil Nadu, India, for a period of four months from March 2021 to June 2021. All consecutive adult patients attending ICTC during the study period for voluntary testing or referred from antenatal clinic or Sexually Transmitted Disease (STD) clinic were included. Patients who were on treatment or follow-up of syphilis were excluded. Pretest counselling session was given and consent was taken for HIV testing. The HIV antibody tests were performed and interpreted according to the National AIDS Control Programme (NACO) guidelines. Rapid Plasma Reagin test (RPR) screening was done. Positive cases were subjected to *Treponema pallidum* Haemagglutination Assay

(TPHA) and Syphilis Rapid Immunochromatographic Test (RICT). The results were analysed with the data systematically entered in Microsoft Excel format and p-value was calculated by Fisher's-exact test using epi info™ software.

**Results:** Among the 400 patients attending the ICTC who were included in the study, six patients had a reactive HIV test. The overall HIV positivity was 1.5% (6/400). The RPR test was positive in 7/400 patients (1.75%). Among the seven patients with RPR positivity, five patients were positive by TPHA and Syphilis RICT. Biological False Positivity (BFP) rate in RPR against TPHA was 28%. Thus the frequency of syphilis among the study population by the confirmatory test TPHA was 1.25%. Among the five confirmed cases of syphilis, four patients were males (80%) and one patient was female gender (20%). Four of the five patients were in the 28-37 year old age group (80%) and one patient was in the 38-47 year age group (20%). The frequency of syphilis was 16.67% among HIV positives (17%) and 1.02% among HIV negatives biological false positivity by RPR was observed in 29% of the cases.

**Conclusion:** HIV and Syphilis co-infection is common and Specific Treponemal tests could contribute to reducing errors due to false positivity by non specific tests like RPR which can be used as a screening test in microbiology laboratories.

**Keywords:** Co-infection, Haemagglutination assay, Integrated counselling and test centre, Rapid test, Sexually transmitted diseases, *Treponema pallidum*

## INTRODUCTION

The World Health Organisation's (WHO's) global health-sector strategy on sexually transmitted infections (2016-2021) has set targets for a 90% reduction in the incidence of *Treponema pallidum* (Syphilis) and *Neisseria gonorrhoeae* (Gonorrhoea) infections between 2018 and 2030 [1]. Studies show that decrease in prevalence of syphilis as well as prevention of perinatal transmission of syphilis gives a hope that syphilis can cease to be a major public health problem, yet limited surveillance of sexually transmitted illnesses in vulnerable population may hinder the further progression towards elimination of syphilis in South east Asia [2]. The presence of syphilis infection increases the chances of transmission of HIV. Syphilis has a negative impact on HIV infection, resulting in increasing viral loads and decreasing CD4 cell counts during syphilis infection [3]. There are many methods like Dark field microscopy, immunofluorescence, and Polymerase Chain Reaction (PCR) for the diagnosis of Syphilis. As microscopy is difficult and culture methods are not available, antibody detection methods are of paramount importance in the diagnosis of syphilis [4]. Syphilis can be detected by two types of tests: non specific Cardiolipin tests like the Venereal Disease

Research Laboratory (VDRL) test, RPR, and specific tests like the TPHA [4].

The TPHA has a high sensitivity for all the stages of disease other than very early primary syphilis [4,5]. These tests use the indirect haemagglutination method to detect antibodies to *Treponema pallidum*. The WHO recommends combining a non treponemal test and a treponemal test for screening and diagnostic purposes [1]. HIV and syphilis co-infection is common and affects different age groups. The aetiological agent of syphilis, namely *Treponema pallidum* may enhance the transmission of the virus, probably through increased incidence of genital ulcers. Detection and treatment of syphilis, therefore, help to reduce HIV transmission [6]. The term "biologically false positive reaction" denotes the reactivity of lipoidal antigens and cardiolipin antigens with the sera from patients who do not have syphilis or other treponematoses. Lynn WA and Lightman S, have observed that biological false positives to serological tests for syphilis may depend on individual immune response, the type of serological test and the disease stage [7].

Though many studies have been done in various parts of the country, not much data (a single study by Santhakumar A et

al., [8] about the seroprevalence of syphilis in the district) is available in Villupuram district which has a predominantly low socio-economical population who are mostly migrant workers with increased risk of contracting STDs. The present study aims to determine the seroprevalence of syphilis, quantify HIV-syphilis co-infection rates, and to determine the proportion of biological false positive reactions in RPR when compared to treponemal tests like TPHA in a rural tertiary care centre in Villupuram district, Tamil Nadu, India.

## MATERIALS AND METHODS

This was a cross-sectional study that was conducted at the Department of Microbiology, Government Villupuram Medical College, Tamil Nadu, India for a period of four months from March 2021 to June 2021. The study was approved by the Institutional Ethics Committee (IEC) (GVMC/IEC/ 2021/5) and informed written consent was obtained from patients.

**Inclusion criteria:** All consecutive adult patients (age >18 years) attending the ICTC for voluntary testing or referred from antenatal or STD clinic were included in the study.

**Exclusion criteria:** Patients who were on treatment or follow-up of syphilis and age <18 years were excluded from the study.

**Sample size:** A minimum sample size of 400 was derived by considering the expected prevalence of Syphilis as confirmed by TPHA as 0.7% (Rajendran P et al.,) [9] with a margin of error of 8% and 95% confidence level.

**Pretest counseling:** Pretest counseling session was given by the counselors regarding the route and mode of transmission, window period, preventive measures, and treatment aspects of HIV. Consent was taken for HIV testing. The confidentiality of the patients was maintained. The patient's demographic details were collected along with their clinical history.

**Sample collection and processing:** 5 mL of venous blood sample was collected in a plain vacutainer, allowed to clot for 10-15 minutes, and centrifuged until the serum was separated. The HIV antibody tests were performed and interpreted according to the NACO guidelines [10]. All samples were subjected to first kit HIV 1 and 2 immunodot assay (Comb AIDS-RS ADVANTAGE ST). Negative samples were declared as negative. If positive, the samples were run in second (Voxpress HIV 1/T rapid immunochromatography) and third (Meriscreen HIV 1-2 WB rapid immunochromatography) kits simultaneously. If two of them were positive, then the result was declared as positive. If, two of them were negative, the test was declared as negative. If one of the two tests was negative, the sample was referred to the State Reference Laboratory (SRL, Chengelpet medical college). The remaining serum was stored in a deep freezer in two aliquots each at -20°C till testing was done. The serum was thawed and brought to room temperature before performing the RPR and TPHA.

**RPR test Qualitative test:** The test was performed according to the manufacturer's instructions provided along with the kit (Biolab Diagnostics, India). Using the sample dispensing device, one drop of RPR reagent was added to one drop of test serum, mixed well and gently rotated on a VDRL shaker for seven minutes. Positive and negative controls were included. The results were observed under a bright light source for clumping of carbon particles and compared with the positive and negative controls.

**RPR Quantitative tests:** The RPR reactive samples were subjected to serial two-fold dilutions from 1:2 to 1:64 according to the kit protocol.

**TPHA:** The reactive samples by qualitative and quantitative RPR tests were subjected to TPHA (Plasmatec Healthcare Ltd, UK). Serum containing antibodies to *Treponema pallidum* react with avian erythrocytes sensitised with pathogenic *Treponema pallidum* (Nichol's strain) and produce visible agglutination. Control cells which are avian erythrocytes not coated with *Treponema pallidum* antigen can detect any non specific reaction. TPHA was taken as the confirmatory test for syphilis.

**Interpretation:** The test was interpreted as follows:

Reaction	Interpretation
Even layer of agglutination	Positive
No agglutination	Negative
Agglutination in the control well	Invalid

**Syphilis Antibody rapid card test (Athenese -Dx-The TRUSTline Syphilis Ab Rapid test):** 150-200 µL or 3-4 drops of serum or plasma were collected in a sample container. When ready to test, the pouch at the notch was opened and the test strip dipped into the specimen for atleast 10 seconds. The strip was then removed from the specimen, and placed on a flat, dry surface. The result was read in 10-15 minutes. Syphilis antibody rapid card test was used for evaluation and not taken as confirmatory.

## STATISTICAL ANALYSIS

Analysis was done with the data systematically entered in Microsoft Excel format and p-value was calculated by Fisher's-exact test using epi info™ software. Statistical significance was considered when the p-value obtained was <0.05. The test findings of the RPR test was compared with the reference method TPHA. Kappa value was used to determine the significance of the agreement between the tests.

## RESULTS

A total of 400 consecutive adult patients attending the ICTC during the study period were included. The male: female:transgender ratio was 70:329:1 with 70 males (17.5%) and 329 females (82.25%) and 1 transgender (0.25%) [Table/Fig-1]. The age of the patients ranged from 19 years to 59 years with a mean age of 38. The commonest age group of patients was 18-27 years (58.25%) followed by 28-37 years (39%), 48-57 years (1.5%) and 38-47 years (1%) [Table/Fig-1]. Among the 400 patients attending the ICTC, 219 patients (54.75%) were antenatal, 76 (19%) patients came for voluntary testing, 105 (26.25%) patients were referred from STD clinic. The two main clinical presentations among the patients were genital itch [n=3(0.75%)] and rashes over body [n=2 (0.5%)]. Majority were asymptomatic [n=395 (98.75%)].

Six patients had a reactive HIV test [Table/Fig-2]. All were male patients. The overall HIV positivity was 1.5% (6 of 400). The frequency of HIV positivity among voluntary ICTC attendees was 5.3% (4 of 76), 1.9% among patients referred from STD (2 of 105), and 0% among antenatal cases. Among the 400 patients, the RPR test was positive in 7 patients (1.75%). Four of seven patients were males (57.14%) and 3 patients were females (42.86%) [Table/Fig-3]. Among the seven patients with RPR positivity, five were positive by Syphilis Rapid card test and TPHA [Table/Fig-4]. Thus the frequency of syphilis among the study population by the confirmatory test TPHA was 1.25% (5/400).

RPR test positivity among ICTC attendees versus STD referrals (n=400) was compared [Table/Fig-5] by Fisher's exact test, two-tailed p-value equals 0.0016. Hence, the association of RPR positivity in STD referrals to ICTC was statistically significant (p<0.05) when compared to RPR positivity in ICTC (Voluntary testing and ANC).

Age (years)	Male (n=70) (17.5%)			Female (n=329) (82.25%)			Transgender (n=1) (0.25%)			Total (n=400)
	Asymptomatic	Genital itch	Rash	Asymptomatic	Genital itch	Rash	Asymptomatic	Genital itch	Rash	
18-27	16	2	-	215	-	-	0	-	-	233 (58.25%)
28-37	45	-	1	110	-	-	0	-	-	156 (39%)
38-47	1	-	1	1	-	-	1	-	-	4 (1%)
48-57	3	1	-	2	-	-	0	-	-	6 (1.5%)
58-67	0	-	-	1	-	-	0	-	-	1 (0.25%)
>67	0	-	-	0	-	-	0	-	-	0

**[Table/Fig-1]:** Age and sex distribution with clinical features of the study population (n=400).

Category of patients	Males (n=70)	Female (n=329)	Transgender (n=1)	Total (n=400)
	HIV positive	HIV positive	HIV positive	HIV positive
ICTC attendees (voluntary testing) (n=76)	4	0	0	4 (5.3%)
STD referrals to ICTCs (n=105)	2	0	0	2 (1.9%)
ANC (n=219)	0	0	0	0
Total (n=400)	6	0	0	6 (1.5%)

**[Table/Fig-2]:** Distribution of HIV status among study population (n=400).

HIV: Human immunodeficiency syndrome; ANC: Antenatal clinic; STD: Sexually transmitted disease; ICTC: Integrated counselling and testing centre

Category of patients	Male	Female	Transgender	Total
ICTC attendees (Voluntary testing)	0	0	0	0
Referred to ICTC from STD clinic	4	2	0	6 (1.5%)
Referred to ICTC from ANC clinic	-	1	-	1 (0.25%)
Total	4	3	0	7 (1.75%)

**[Table/Fig-3]:** Overall RPR positivity among various categories of study patients (n=400).

ANC: Antenatal clinic; STD: Sexually transmitted disease; ICTC: Integrated counseling and testing centre

	RPR positive	Syphilis antibody Rapid immunochromatographic test positive	TPHA positive
ICTC attendees (voluntary testing)	-	-	-
Referred to ICTC from STD clinic	6	5	5
Referred to ICTC from ANC clinic	1	-	-

**[Table/Fig-4]:** Distribution of patients with positive RPR, Syphilis RICT, and TPHA (n=7).

RPR: Rapid plasma reagin test; TPHA: Treponema pallidum haemagglutination test; ANC: Antenatal clinic; STD: Sexually transmitted disease; ICTC: Integrated counselling and testing center

	RPR negative	RPR positive	p-value
ICTC attendees (voluntary testing+ANC)	294	1	p=0.0016 (Significant)
STD referrals to ICTC	99	6	

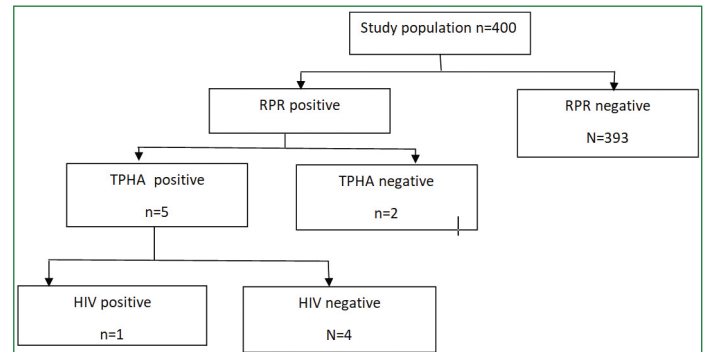
**[Table/Fig-5]:** Comparison of RPR test positivity among ICTC attendees versus STD referrals (n=400).

RPR: Rapid plasma reagin test; ANC: Antenatal clinic; STD: Sexually transmitted disease; ICTC: Integrated counselling and testing centre

The RPR test with the confirmatory test TPHA [Table/Fig-6] was evaluated. The sensitivity of RPR -100%; specificity-99.4%, Positive Predictive value-71%, Negative Predictive value-100%, kappa value-0.81 (Perfect agreement).

Among the five confirmed cases of syphilis, four patients were males (80%) and one patient was female gender (20%). Four of five patients were in the 28-37-year-old age group (80%) and one patient was in the 38-47 year age group (20%).

Two cases out of seven which were RPR positive were negative by TPHA. Hence, the Biological false positivity rate of the RPR test was noted as 28%. The association of gender with syphilis was compared. By Fisher's exact test, the two-tailed p-value equals 0.0019 [Table/



**[Table/Fig-6]:** Biological false positivity rate in RPR against TPHA.

RPR: Rapid plasma reagin test; TPHA: Treponema pallidum haemagglutination test

Fig-7]. Hence, the association of TPHA/ICT positivity in males as compared to females was statistically significant ( $p < 0.05$ ). The risk ratio is 18.8 inferring that males are more at risk than females of acquiring syphilis. But, this may be because more females have been enrolled in the study. Of the 400 patients who were enrolled in the study, six were HIV positive. The frequency of syphilis among HIV-positive patients was 16.67% (1 of 6 patients) [Table/Fig-8]. The frequency of syphilis among HIV-negative patients was 1.02%.

Sex	Syphilis positive	Syphilis negative	p-value
Males	4	66	p=0.0019 (Significant)
Females	1	328	

**[Table/Fig-7]:** Association of gender and syphilis (n=5).

	Patients with syphilis	Patients without syphilis
HIV positive	1	5
HIV negative	4	390

**[Table/Fig-8]:** Association of syphilis and HIV status (n=400).

## DISCUSSION

India has successfully achieved the 6<sup>th</sup> Millennium Development Goal of halting the HIV epidemic [11]. National adult (15-49 years) HIV prevalence was estimated at 0.22% in 2020; 0.23% among males, and 0.20% among females [11]. There is a decline of about 33.3% in the national adult prevalence from an estimated peak level of 0.33% in 2010 to 0.22% in 2020 [11]. The current study which was conducted on 400 ICTC attendees observed an HIV seropositivity of 1.5% [Table/Fig-2]. Anjana N et al., have reported an HIV seropositivity of 3.78% among ICTC attendees in Central India from 2011 to 2015 [12]. Chougale R and Shinde P, have reported an HIV seropositivity of 1.24% among ICTC attendees between 2013-and 2017 from Kolhapur, India [13]. The maximum number of attendees and seropositive patients were young and middle aged adults [Table/Fig-1], as reported elsewhere in the country [14].

There are a wide variety of diagnostic modalities for the diagnosis of syphilis. Every test has its limitations and using a single test for diagnosis has its own limitations. The sensitivity of non-treponemal tests varies with the stage of syphilis. The diagnosis of syphilis is as follows: first screening with a non treponemal test such as the RPR test and then confirmation using a treponemal test such as



TPHA. All non treponemal serologic tests measure antibodies to cardiolipin. A single test of non treponemal antibodies like RPR and VDRL should not be considered confirmative because it detects only the reaginic antibodies which do not conclusively prove the active stage of the disease [15]. Many physiological conditions such as pregnancy and certain acute and chronic conditions (such as Systemic lupus erythematosus, Rheumatoid disease, Rheumatic fever) may give a biologically false positive report. Tests may also show false negativity in late and latent syphilis [15]. RPR positivity must be confirmed by specific treponemal tests such as TPHA, as mentioned in the national Sexually Transmitted Infections (STI) management guidelines [16]. The overall RPR positivity in the study population was 1.75%. The confirmation of RPR positivity by TPHA and syphilis rapid card test revealed a prevalence of 1.25% [Table/Fig-3]. The prevalence of syphilis in the community of Tamil Nadu as per RPR positivity is reported as 2.7% as against 0.7% by TPHA as observed by Rajendran P et al., [9]. RPR positivity was 0.61% as observed by NACO [11]. Present study results (1.25%) are slightly higher, but a further continuation of the study may reflect the exact situation. The association of TPHA/ICT positivity in STD referrals to ICTC was found to be statistically significant ( $p < 0.05$ ) when compared to TPHA/ICT positivity in ICTC attendees (Voluntary testing and antenatal care) [Table/Fig-4].

Biological false positivity was observed as 2 of 7 positive cases (29 %) in present study. 71% of RPR reactive results were confirmed by TPHA and IgM rapid immunochromatography test in present study. This was comparable to 81.6% of RPR reactive results that were confirmed by the TPHA test in a study by Arti BN and Mihir RD, [17] and 73.2% of RPR reactive results that were confirmed by the TPHA test in the study of Dumre SP et al., [18].

TPHA and rapid immunochromatography positive cases with RPR negative results were found in two patients in the present study, an antenatal mother and a patient with rheumatoid arthritis on steroids. Reaginic antibodies are produced by patients with syphilis and also by patients with diseases like leprosy, tuberculosis, chancroid, leptospirosis, malaria, lymphogranuloma venereum, autoimmune diseases, and factors like old age, pregnancy, and recent immunisation [4,19]. The sensitivity of RPR according to present study was calculated to be 100% and the specificity was 99.4%. RPR has a positive predictive value of 71% whereas the negative predictive value was 100 %. The kappa value for the RPR test was calculated to be 0.8188 showing almost perfect agreement with the standard test [Table/Fig-6]. This was comparable with the study by Calonge N [20], where the sensitivity of the RPR and VDRL tests are estimated to be 78% to 86% for detecting primary syphilis infection, 100% for detecting secondary syphilis infection, and 95% to 98% for detecting latent syphilis infection. The syphilis IgM immunochromatography was evaluated along with TPHA test and was found to be in perfect agreement with the confirmatory test-TPHA. However, the agreement between TPHA and Rapid immunochromatography has to be assessed with a larger sample size and hence no conclusion could be drawn about the utility of the same at this stage. Biological false positivity rate of the RPR test was noted as 28% in our study.

Males were affected in 80% cases and females were affected in 20% cases by TPHA [Table/Fig-7]. The association of TPHA/ICT positivity in males as compared to females was found to be statistically significant ( $p < 0.05$ ). The risk ratio is 18.8, indicating that males are at a higher risk than females of acquiring syphilis. But, this may be subject to the fact that more females have been enrolled in the study as compared to males. This correlates with the study by Arti BN and Mihir RD, which shows 56.3% of males were more affected than females [17].

Individuals with STI/Reproductive Tract infections have a significantly higher chance of acquiring and transmitting HIV. Of the 400 who

were enrolled in the study six were HIV positive one was co-infected by HIV and syphilis, i.e., TPHA positivity was 16.6% among HIV positives (17%) and 1.02% among HIV negatives. [Table/Fig-9] shows literature search revealed a varying prevalence of syphilis among HIV infected patients [8,21-24].

Name of author [Reference no]	Place of study	Year of publication	Syphilis	HIV	Syphilis and HIV Co-infection
Gamba EP et al., [21]	Central african republic	2013	7.6%		9.7% of HIV infected
Behara SK and Bindu Satti SA [22]	India	2021			10% of those HIV infected
Santhakumar A et al., [8]	Villupuram	2021		0.3%	
Sumana MN and Kishore A, [23]	Karnataka	2016	0.29%	4.71%	7% of those HIV infected
Bhattar S et al., [24]	New Delhi	2014	5.45%	6.36%	1.36% of total
Present study	Villupuram	2022	1.25%	1.5%	16.67% of those HIV infected

[Table/Fig-9]: Shows comparison with different studies [8,21-24].

Also, a 23% HIV- syphilis co-prevalence rate were seen in a population-based study in HIV positive Andhra Pradesh, India [25]. Bourouache M et al., reported in their study that syphilis was prevalent in 16.42% in Morocco [26]. Haule A et al., reported a prevalence of syphilis in HIV-infected patients of 9.6% in Tanzania [27]. Santos AM et al., reported a prevalence of 18.4% among HIV-infected patients in Brazil [28]. Khan S et al., reported a prevalence of 6.5% syphilis among the HIV-positive individuals screened for syphilis in South India between 2006 and 2008 [29]. A facility-based cross-sectional study conducted utilising National HIV Sentinel Surveillance of Meghalaya, January-March 2017 revealed a prevalence of 1.03% among antenatal women [30]. Present study gives a slightly higher percentage of Syphilis-HIV coinfection (16.67%). This study attempted to measure the prevalence of two major STD syphilis and HIV and their co-infection in an ICTC which included population with a variable risk (high risk STD attendees to patients who were asymptomatic and Antenatal women who had a comparatively lower risk). This data will help us to strengthen STI (especially syphilis) surveillance in Villupuram district and will take us a step nearer to reducing syphilis burden in the rural community of this district.

### Limitation(s)

The limitations of the study were the small sample size and a lack of follow-up of the patients as this was a short-term study.

### CONCLUSION(S)

The overall prevalence of syphilis among the ICTC attendees in this study was 1.25%, HIV prevalence was 1.5% and the rate of HIV syphilis coinfection was 16.67%. Biological false positivity by RPR was observed in 29% of the cases. Specific Treponemal tests could contribute to reducing errors due to false positivity by non specific tests like RPR. RPR test is a good choice as a screening test in microbiology laboratories. But non specific tests like RPR have their limitations and specific tests like TPHA must be used for all RPR positive cases.

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**PARTICULARS OF CONTRIBUTORS:**

- Associate Professor, Department of Microbiology, Government Villupuram Medical College, Villupuram, Tamil Nadu, India.
- Associate Professor, Department of Microbiology, Stanley Medical College, Chennai, Tamil Nadu, India.
- Professor, Department of Immunology, Madras Medical College, Chennai, Tamil Nadu, India.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**

Dr. K Kavitha,  
No.32 A Ther Pillaiyar Koil Street Salamedu Villupuram, Tamil Nadu, India.  
E-mail: sahanathirumurugan@gmail.com

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