The first performance report for the Bio-Rad Dx CT/NG/MG assay for simultaneous detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* in urogenital samples

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**ABSTRACT**

We evaluated the clinical performance of the Bio-Rad Dx CT/NG/MG assay for the detection of *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Neisseria gonorrhoeae* in urogenital samples in comparison with the Roche COBAS® TaqMan® CT assay for *C. trachomatis* and an in-house TaqMan PCR assay for *M. genitalium*. Swab specimens were cultured for *N. gonorrhoeae*. In this prospective study, urogenital samples were obtained from symptomatic and asymptomatic patients attending the sexually transmitted disease clinic of Bordeaux, France, from January to April 2010. A total of 658 clinical specimens (259 male and 180 female urines, 191 vaginal, 21 endocervical and 7 urethral swabs) from 453 patients were analyzed. The prevalence of *C. trachomatis* and *M. genitalium* infections was 8.1% (21/260) and 1.9% (5/260) in men and 10.4% (20/193) and 2.1% (4/193) in women, respectively. The Bio-Rad Dx CT/NG/MG clinical sensitivity was 100% for *C. trachomatis* and *M. genitalium* in men and women. In male urine, the clinical specificity was 99.6% for *C. trachomatis* and 100% for *M. genitalium*. In women, the specificity was 99.5% for swabs and 100% for urines for detecting *C. trachomatis* and *M. genitalium*. All seven *N. gonorrhoeae* PCR-positive samples were also positive by culture. Patients were co-infected in 5/57 cases (8.8%), with *C. trachomatis* and *M. genitalium* in three cases, and *C. trachomatis* and *N. gonorrhoeae* in two cases. In conclusion, the Bio-Rad Dx CT/NG/MG assay can be recommended for the simultaneous detection of *C. trachomatis*, *M. genitalium* and *N. gonorrhoeae* in urogenital specimens of symptomatic and asymptomatic individuals.

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1. Introduction

*Chlamydia trachomatis* infections are among the most prevalent bacterial sexually transmitted infections (STIs) worldwide (Peipert, 2003; Manavi, 2006; Bébéar and de Barbeyrac, 2009). Chlamydial infection may cause urethritis, epididymitis, cervicitis, pelvic inflammatory disease (PID), ectopic pregnancy and tubal infertility (Manavi, 2006). *Mycoplasma genitalium* is a sexually transmitted organism associated with acute and chronic non-gonococcal urethritis in men. Existing data on infections in women suggest that *M. genitalium* is associated with urethritis, cervicitis and pelvic inflammatory disease (Manhart et al., 2011; Taylor-Robinson and Jensen, 2011). Like *C. trachomatis* infection (Peipert, 2003), *M. genitalium* infection is often asymptomatic (Falk et al., 2005; Ross et al., 2009). Screening for *C. trachomatis* and *M. genitalium* infections within patients attending sexual health clinics is needed, not only to identify infected symptomatic individuals for the diagnosis and management of their infection, but also to identify asymptomatic individuals who serve as reservoirs for infection. Detection of *M. genitalium* is hampered by the absence of a commercially available diagnostic test. Persons with persistent PID or clinically significant persistent urethritis or cervicitis should be tested for *M. genitalium* (Manhart et al., 2011). It is all the more important since the current treatment for NGU and cervicitis does not always eradicate this organism (Falk et al., 2003; Mena et al., 2009). Nucleic acid amplification tests (NAATs) are the tests of choice for the diagnosis of genital infections because of their high sensitivity, specificity and suitability for various types of samples, including vulvovaginal swabs and first-catch urine (FCU) (Semeniuk et al., 2002; Jensen et al., 2004a, 2004b; Bébéar and de Barbeyrac, 2009), non-invasively self-collected specimens, which are ideal for high-throughput asymptomatic identification (Levett et al., 2008). Because co-infections are common, a number of NAATs for the detection of *C. trachomatis* employ,
or are pursuing, multiplex formats to reduce the risk of underreporting infections. Recently, an increasing number of laboratories are offering combinatorial NAATs for the diagnosis of both *C. trachomatis* and *Neisseria gonorrhoeae* infections (Gaydos et al., 2010; Hopkins et al., 2010; Rockett et al., 2010; Kerndt et al., 2011). With the use of non-commercial PCR testing, it has been shown that *M. genitalium* is a sexually transmitted pathogen (Jhorth et al., 2006) and that the spectrum of diseases in both males and females is similar to that caused by *C. trachomatis* and *N. gonorrhoeae* (Manhart et al., 2011; Taylor-Robinson and Jensen, 2011). The Bio-Rad company has included an *M. genitalium* target in their assays to facilitate the detection of the three most prevalent bacterial STIs worldwide. The Bio-Rad Dx CT/NG/MG assay performs manual DNA extraction, contains an internal control and employs a real-time multiplex PCR method that comprises reactions for *C. trachomatis*, *N. gonorrhoeae* and *M. genitalium*. Here, we investigated the clinical performance of the Bio-Rad Dx CT/NG/MG assay for the detection of these three pathogens using specimens obtained from symptomatic and asymptomatic male and female subjects from a French sexually transmitted disease (STD) clinic. We compared the results to those obtained with the Roche COBAS® TaqMan® CT assay for *C. trachomatis*, an in-house TaqMan PCR assay for *M. genitalium* and a culture for *N. gonorrhoeae*.

2. Materials and methods

2.1. Patient population

Consecutive patients attending the STD clinic “Maison Départementale de la Santé” in Bordeaux, France, were recruited in the study from January to April 2010. Subjects meeting the following criteria were considered eligible for the study: (i) Inclusion criteria: the patient was 18 years of age or older, affiliated with the French Social Security System and accepted that additional samples would be taken as indicated in the information letter. The individual signed and dated a non-opposition and accepted that additional samples would be taken as indicated in the information letter. The individual signed and dated a non-opposition and accepted that additional samples would be taken as indicated in the information letter. (ii) Exclusion criteria: Menstruating women, patients who reported using or having completed antimicrobial therapy within 21 days of enrollment in the study and patients having voided within 1 h of specimen collection.

2.2. Specimen collection

2.2.1. Female subjects

Five specimens were collected from each symptomatic female subject: two self-collected vaginal swabs (one with the routine Copan® regular flocked swab and one with the Bio-Rad flocked swab in its transport system), two clinician-collected endocervical swabs (one using the routine Copan® flocked swab in the Universal Transport Medium (UTM) and one using the Bio-Rad collection kit) and a FCU. Three specimens were collected from each asymptomatic female subject: two self-collected vaginal swabs (one with the routine Copan® regular flocked swab and one with the Bio-Rad flocked swab in its transport system) and a FCU.

2.2.2. Male subjects

Three specimens were collected for each symptomatic male subject: two urethral swabs (one using Copan® flocked swab in the UTM routine transport medium and one using the Bio-Rad collection kit) and a FCU. A sole FCU sample was collected for each asymptomatic male subject.

2.3. Bio-Rad Dx CT/NG/MG assay

Before the extraction step, urine samples were frozen for one night at −20 °C. The nucleic acid extraction was performed manually in accordance with the Bio-Rad Dx real-time CT/NG/MG testing protocol. For each sample, 1 mL of the swab sample in the Bio-Rad transport system or thawed urine was centrifuged, and a volume of 410 μL of the Bio-Rad lysis mix containing an internal control was added to the pellet; samples were then vortexed, warmed to 100 °C and centrifuged again. Nucleic acid extracts contained in the supernatant were amplified by the multiplexing Bio-Rad Dx real-time CT/NG/MG assay, according to the manufacturer’s instructions, on the Bio-Rad thermocycler Dx Real-Time System associated with the IVD software. The results were described as positive or negative and were considered invalid by an algorithm within the IVD software.

2.4. Comparison assays

2.4.1. DNA extraction

Before the extraction step, the dry Copan® regular flocked swab was re-suspended in 1 mL of a home-prepared 2SP medium solution (sucrose 0.2 M, K2HPO4 15 mM, KH2PO4 6 mM and water pH = 7). Urine samples were frozen at −20 °C overnight and 500 μL of thawed urine was centrifuged; 200 μL of lysis buffer from the MagNaPure LC DNA isolation kit I (Roche Diagnostics, France) was then added to the pellet. Before the extraction step, a volume of 3 μL of the internal control “DNA extraction and PCR Control inhibition” (Diagnode, Belgium) was added to 200 μL of pre-treated urine or swab samples. Nucleic acid extraction was performed with the MagNaPure LC DNA isolation kit I on the extractor MagNaPure LC (Roche Diagnostics, France) according to the manufacturer’s instructions.

2.4.2. Detection of *C. trachomatis*

This was performed with the Roche COBAS® TaqMan® CT test v2.0 on the COBAS TaqMan 48 (Roche Diagnostics, France), according to the manufacturer’s instructions.

2.4.3. Detection of *M. genitalium*

This was performed using an in-house TaqMan PCR assay targeting the MgPA gene (Jensen et al., 2004a) with detection of the internal control added during the extraction step on an ABI Prism 7000 (Applied Biosystems, USA).

2.4.4. Detection of *N. gonorrhoeae*

This was performed on swab samples collected in UTM transport medium using a culture technique on gelose PolyViteX Chocolate VCAT (bioMerieux, France) with a first reading at 24 h and a second at 5 days.

2.5. Data analysis

Specimens that were tested positive by both the Bio-Rad Dx real-time CT/NG/MG assay and the reference test were considered consensus-positive for *C. trachomatis* and/or *N. gonorrhoeae* and/or *M. genitalium*. Similarly, specimens that were tested negative by both assays were considered consensus-negative. Clinical sensitivity and clinical specificity of *C. trachomatis* and *M. genitalium* were calculated on the basis of the infection status and by specimen type. Positive- and negative-percent agreements, along with their 95% confidence intervals, and overall-percent agreement were calculated based on the initial results (Simel et al., 1991). A symptomatic or an asymptomatic female subject and a symptomatic male subject were defined as infected by *C. trachomatis* or *M. genitalium* if at least two positive results were reported from either of the two assays that were performed on every sample from the subject. For the asymptomatic men, for which only one specimen was available, any discrepant sample underwent repeat testing using both Bio-Rad and reference methods. For these samples, initial and retest results were grouped together to determine the patient status. The asymptomatic men were defined as infected by *C. trachomatis* or *M. genitalium* if at least two positive results were reported.

A subject was defined as infected with *N. gonorrhoeae* if the culture result was positive.
3. Results

3.1. C. trachomatis results

A total of 453 male and female, asymptomatic and symptomatic subjects were enrolled. Of the 193 female subjects included, 166 (86%) were asymptomatic and 27 (14%) were symptomatic. Of the 260 male subjects enrolled, 236 (90.8%) were asymptomatic and 24 (9.2%) were symptomatic. A total of 658 specimens were collected, 392 for female subjects (180 urine samples, 191 vaginal swabs and 21 endocervical swabs) and 266 for male subjects (259 urine samples and 7 urethral swabs).

The first analysis of the C. trachomatis results on 658 specimens found that eight specimens (2 male and 2 female urine samples, 1 endocervical and 3 vaginal swabs) provided discrepant results (Tables 1 and 2). Positive and negative percent agreements between the Bio-Rad Dx CT/NG/MG assay and the COBAS® TaqMan® CT Roche assay were 95.4% (95% CI, 87.4%–98.4%) and 99.2% (95% CI, 98.0%–99.6%), respectively, with an overall agreement of 98.8% (650/658). After analyzing the discrepant results, the Bio-Rad assay still yielded 2 false-positive test results (one male urine and one cervical sample) (Tables 1 and 2). The clinical sensitivity of the Bio-Rad Dx CT/NG/MG assay for C. trachomatis was 100%, regardless of the type of the specimen tested, except for male urethral swabs for which the low number of specimens excluded any sensitivity evaluation. The clinical specificity ranged from 99.5% to 100% based on the specimen type (Table 3). Overall, the C. trachomatis PCR TaqMan assay was 56.2% (95% CI, 33.2%–76.9%) and 99.7% (95% CI, 98.5%–99.9%), respectively, with an overall agreement of 98.6% (649/658). After analyzing the discrepant results, the Bio-Rad assay still yielded one false-positive test result on a vaginal swab sample. The clinical sensitivity of the Bio-Rad Dx CT/NG/MG assay for M. genitalium was 100%, regardless of the type of the specimen tested, except for the urethral swabs. The clinical specificity ranged from 99.5% to 100% based on the specimen type (Table 3). Overall, the M. genitalium prevalence in females was 2.1% (4/193), 1.8% (3/166) among asymptomatic and 3.7% (1/27) among symptomatic.

3.2. M. genitalium results

The first analysis of the M. genitalium results on 658 specimens found that nine specimens (4 vaginal swabs, 3 female and 2 male urine samples) provided discrepant results (Tables 1 and 2). Positive and negative percent agreements between the Bio-Rad Dx CT/NG/MG assay and the in-house M. genitalium PCR TaqMan assay were 56.2% (95% CI, 33.2%–76.9%) and 99.7% (95% CI, 98.5%–99.9%), respectively, with an overall agreement of 98.6% (649/658). After analyzing the discrepant results, the Bio-Rad assay still yielded one false-positive test result on a vaginal swab sample. The clinical sensitivity of the Bio-Rad Dx CT/NG/MG assay for M. genitalium was 100%, regardless of the type of the specimen tested, except for the urethral swabs. The clinical specificity ranged from 99.5% to 100% based on the specimen type (Table 3). Overall, the M. genitalium prevalence in females was 2.1% (4/193), 1.8% (3/166) among asymptomatic and 3.7% (1/27) among symptomatic.

3.3. N. gonorrhoeae results

Of the 658 specimens, 13 specimens (1 endocervical, 2 vaginal and 2 female urine samples, 3 urethral swabs and 5 male urine samples) from 7 patients (2 females and 5 males) provided PCR–positive results for N. gonorrhoeae by the Bio-Rad Dx CT/NG/MG assay. In total, we observed 100% total agreement of the 219 swab specimens tested by culture technique and the Bio-Rad Dx CT/NG/MG assay. Overall, the N. gonorrhoeae prevalence in females was 1% (2/219), 0.6% (1/166) among asymptomatic and 3.7% (1/27) among symptomatic. Moreover, the prevalence was 1.9% (5/260) in males, 0.4% (1/236) among asymptomatic and 16.7% (4/24) among symptomatic patients.

Finally, of the 57 infected cases (41 C. trachomatis, 9 M. genitalium, 7 N. gonorrhoeae), co-infections occurred in three patients with C. trachomatis and M. genitalium and in two patients with C. trachomatis and N. gonorrhoeae.

4. Discussion

In this study, the results of the Bio-Rad Dx CT/NG/MG assay agreed with the established methods. Overall, sensitivities of 100% and specificities of 99.5% and 100% for C. trachomatis and M. genitalium, respectively, provided excellent results. The performance of the Bio-Rad Dx CT/NG/MG assay was not affected by the presence or absence of symptoms in the patients, suggesting that the test could be suitable for routine screening of populations. The use of NAATs for the detection of C. trachomatis is well established; NAATs are increasingly being used for the detection of N. gonorrhoeae and M. genitalium, as well, particularly as emphasis shifts toward the use of non-invasive specimen types (Skidmore et al., 2006; Alloba et al., 2007; Shipitsyna et al., 2010). The ability to use FCU specimens has made it far easier to screen asymptomatic individuals in places other than traditional clinical settings; it has also made it possible to perform studies to determine the population-based prevalence and even the incidence of these infections. Several companies now supply NAAT platforms that can screen urine samples for both C. trachomatis and N. gonorrhoeae in the same reaction (Gaydos et al., 2010; Hopkins et al., 2010; Rockett et al., 2010; Kerndt et al., 2011). The Bio-Rad company has been the first to include a M. genitalium target in a multiplex real-time PCR assay and we report the first evaluation of this commercialized test in clinical practice. M. genitalium is an emerging cause of STIs in the United States (Manhart et al., 2007) and has been implicated in urogenital infections of men and women around the world. Following the firm establishment that M. genitalium causes NGU in men and is a cause of STI, many studies have now found significant associations with lower and upper reproductive tract disease in

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Table 1
Comparison of C. trachomatis and M. genitalium test results for male specimens.

<table>
<thead>
<tr>
<th>Males</th>
<th>Number of Patients (n = 260)</th>
<th>Bio-Rad Dx CT/NG/MG results</th>
<th>Reference test results</th>
<th>Specimen Bio-Rad status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine (n = 259)</td>
<td>Urethral swabs (n = 7)</td>
<td>Urine (n = 259)</td>
</tr>
<tr>
<td>C. trachomatis</td>
<td>231</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
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<td>ND</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+/+ +/−</td>
<td>ND</td>
<td>−/−</td>
</tr>
<tr>
<td>M. genitalium</td>
<td>246</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>+</td>
<td>ND</td>
<td>+</td>
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<td>6</td>
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<td>−</td>
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<td>ND</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>−/−/−</td>
<td>ND</td>
<td>+/+/−</td>
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</tbody>
</table>

ND, not determined; FP, false-positive; TN, true-negative; TP, true-positive.

* Repeated test.

196

Table 2
Comparison of C. trachomatis and M. genitalium test results for female specimens.

<table>
<thead>
<tr>
<th>Specimen status</th>
<th>Bio-Rad Dx CT/NG/MG performance</th>
<th>Sens. (%) [95% CI]</th>
<th>Spec. (%) [95% CI]</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. trachomatis</td>
<td>Male urine (n = 259)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos 21/1</td>
<td>100 [84.5–100]</td>
<td>99.6 [97.6–99.9]</td>
<td>100</td>
<td>95.2</td>
<td></td>
</tr>
<tr>
<td>Neg 0/237</td>
<td>NA</td>
<td>100 [64.6–100]</td>
<td>NA</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Urethral swabs (n = 7)</td>
<td>Pos 0/0</td>
<td>100 [83.2–100]</td>
<td>100 [97.7–100]</td>
<td>100</td>
<td>95.8</td>
</tr>
<tr>
<td>Female urine (n = 180)</td>
<td>Pos 19/0</td>
<td>100 [85.7–100]</td>
<td>100 [97.1–99.9]</td>
<td>100</td>
<td>95.8</td>
</tr>
</tbody>
</table>

M. genitalium

<table>
<thead>
<tr>
<th>Specimen status</th>
<th>Bio-Rad Dx CT/NG/MG assay</th>
<th>Male urine (n = 259)</th>
<th>5/0</th>
<th>100 [56.5–100]</th>
<th>100</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>5/0</td>
<td>NA</td>
<td>100 [98.5–100]</td>
<td>NA</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Neg</td>
<td>0/254</td>
<td>NA</td>
<td>100 [64.6–100]</td>
<td>NA</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Female urine (n = 180)</td>
<td>Pos 1/0</td>
<td>100 [20.6–100]</td>
<td>100 [97.6–100]</td>
<td>100</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Performance characteristics of the Bio-Rad Dx CT/NG/MG assay for the detection of C. trachomatis and M. genitalium in urine and swab specimens after discrepant result analysis.

ND, not determined; FP, false-positive; TN, true-negative; TP, true-positive.

women (Manhart et al., 2011; Taylor-Robinson and Jensen, 2011). Overall, published data suggest that the pathogenicity of M. genitalium appears to be somewhat similar to that of C. trachomatis (Oakeshott et al., 2010; Walker et al., 2011). Because traditional diagnostic methods, such as culture and serology, are not suitable for routine diagnosis of M. genitalium, identification of infected individuals has to be based on molecular tools and commercial assays are needed. The accurate identification of etiological agents of STI is important to establish treatment recommendations. Indeed, antibiotic treatments that are effective against C. trachomatis appear to be also usually effective in NGU, tetracyclines and azithromycin in the doses used for C. trachomatis do not consistently eradicate M. genitalium. Furthermore, antibiotic treatment recommended for PID are primarily targeted towards C. trachomatis and N. gonorrhoeae to which less than half PID cases can be attributed (Ness et al., 2002). The few studies evaluating the effectiveness of standard regimens recommended for women with M. genitalium PID (Haggerty, 2008; Manhart et al., 2011) suggest that they are ineffective against M. genitalium. Thus it is important to test for M. genitalium infection in the patient with persistent symptoms or treatment failure. Indeed, successful treatment of M. genitalium infection in female patients is of particular importance because prolonged inflammation at upper genital tract sites might lead to significant reproductive tract morbidity and infertility.

Although assay sensitivity and specificity are important considerations when evaluating the reliability of a kit, additional criteria such as applicability, ease of use, and handling time and cost should not be disregarded. The Bio-Rad Dx CT/NG/MG assay showed a simple and short workflow sequence. It allowed prompt and specific results to be validated through the use of an internal extraction and amplification control. The ease of use of the semi-automated sample preparation and the rapid workflow are also conducive to efficient laboratory handling, and the time to results is approximately 4.5–5 h. This included manual extraction (60–100 min), new run set-up (20 min) and PCR (approximately...
Bacterial agents responsible for STIs as well as an internal control using only one real-time PCR reaction and the double-stranded probes technology. This new assay provides clinicians and laboratories with one of the necessary tools to significantly reduce the prevalence of *C. trachomatis* and *N. gonorrhoeae* infections in sexually active men and women, as well as to prevent their costly and serious sequelae. In this study, *C. trachomatis* was the most prevalent STI identified, followed by *M. genitalium* and *N. gonorrhoeae*. Of the asymptomatic patients, the prevalence of *N. gonorrhoeae* remained very low at approximately 0.5%. This test also allowed for the detection of co-infections with these three bacteria. In our study, 8.8% of the infected patients (5/57) presented with co-infection of *C. trachomatis* and *M. genitalium* and two cases presented with both *C. trachomatis* and *N. gonorrhoeae* infections. Other microorganisms, such as *Trichomonas vaginalis*, may play an important role in urethritis in men and should be considered in approach to therapy (Schwebke et al., 2011). Multiplex PCR for the simultaneous detection of STI organisms begins to be introduced as the Seeplex STD6 ACE detection for the diagnosis of six genital pathogens, among whom *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis* (Lee et al., in press).

In conclusion, our study showed that the Bio-Rad Dx CT/NG/MG assay proved to be highly suitable for high-throughput identification of *C. trachomatis*, *M. genitalium* and *N. gonorrhoeae* in urogenital specimens. The ability of the assay to simultaneously detect these three pathogens supports the use of the Bio-Rad assay in large-scale screening programs. Moreover, the availability of a commercial NAA that includes *M. genitalium* as well as *C. trachomatis* and *N. gonorrhoeae* would enable further study of the relationships between these organisms in the etiology of STI and sequelae.

**Transparency declaration**

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**References**


