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Detection of Sexually Transmitted Diseases Using the STD Direct Flow Chip in Jakarta Special Region, Indonesia

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Abstract – Sexually Transmitted Diseases (STDs) remain a major global health concern, with millions of new cases annually. Traditional diagnostic methods, which require separate testing for different pathogens, are time-consuming and costly, hindering disease control efforts. The increasing prevalence of co-infections further emphasizes the need for simultaneous pathogen detection. This study evaluates the effectiveness of the HybriSpot 12 Manual platform and the STD Direct Flow Chip kit in detecting multiple STD pathogens in DKI Jakarta and analyzes infection distribution patterns by gender. Multiplex PCR and hybridization techniques successfully detected 11 pathogens simultaneously, including 8 bacteria, 2 viruses, and 1 protozoon. Among 432 patient samples tested at IMOQ LAB, 88 men and 73 women had single infections, 46 men and 48 women had co-infections, while 120 men and 53 women tested negative. These findings highlight the necessity of efficient diagnostic techniques to enhance STD surveillance and control efforts.

Keywords: hybridization, molecular diagnostics, pathogens, PCR, sexually transmitted diseases

1. Introduction

Sexually Transmitted Diseases (STDs) represent a serious and ongoing public health issue worldwide. According to data from the World Health Organization (WHO) in 2019, millions of new cases of STDs are reported annually, underscoring the urgent need for effective diagnostic and therapeutic interventions [1]. Traditional methods such as microbiological cultures, serological tests, microscopic examinations, and Gram staining, along with other staining techniques used for detecting STDs, often involve time-consuming and costly processes due to the requirement of separate tests for identifying various pathogens. Globally, sexually transmitted infections (STIs) are a significant public health concern, with an estimated more than one million people infected daily. According to the WHO report (2023), more than 374 million new sexually transmitted infections occur each year worldwide, with the highest cases coming from chlamydia, gonorrhea, syphilis, and trichomoniasis [2]. In Indonesia, cervical cancer has a relatively high prevalence, largely driven by human papillomavirus (HPV) infection, which significantly contributes to various types of cancer [3].

Sexually transmitted infections (STIs) are infections primarily transmitted through sexual contact [4]. Over thirty types of pathogens are known to spread through sexual activity. Common STIs include gonorrhea, chlamydia, trichomoniasis, genital herpes, HPV infections, hepatitis B, and syphilis [5]. Research by Irwan (2021) identified a correlation between high-risk sexual behavior and the occurrence of STIs [6]. Young individuals, particularly those aged 16-24, are considered more at risk of contracting STIs compared to older adults. WHO estimates that 20% of people living with

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HIV/AIDS are in their twenties, and one in twenty teenagers contracts an STI annually. STIs have a profound impact on sexual and reproductive health globally. Consequently, STIs can lead to infertility, pregnancy complications, developmental disorders, cancer, and an increased susceptibility to human immunodeficiency virus (HIV) [7].

Based on UNAIDS data in 2023, the rate of HIV infection in the young age group is still very worrying. Data globally shows that every week about 4000 adolescents and young women aged 15-24 are infected with HIV with 3100 cases occurring in sub-Saharan Africa. In addition, the prevalence of HIV in the young adult population (ages 15-49) averages 0.8% globally, with higher rates in certain at-risk groups [8]. According to data from UNFPA and WHO, one in twenty teenagers contracts an STI annually, highlighting that STIs remain prevalent among adolescents [9]. This may be due to a lack of knowledge about STIs and their associated risks among adolescents. The prevalence rates of STIs in Indonesia vary by region. No direct link has been established between rising STI incidence and high-risk behaviors. High-risk behaviors are those that increase a person's likelihood of contracting diseases. High-risk groups include men and women aged 20-34, women aged 16-24, both genders aged 20-24, travelers, commercial sex workers, drug addicts, and homosexual individuals [7]. Among HIV/AIDS patients at the Melati Clinic of Dr. Soedarso Hospital in Pontianak, there were five cases of HIV-syphilis coinfection, according to research by David Proyono, Diana, and Natalia. The HIV-syphilis coinfected patients were males aged 23 to 29, with heterosexual transmission being the most common route. Private sector employment was the most common occupation. In a previous study, the relationship between syphilis and HIV/AIDS, Muhammad Caesario and Jundi Fathan found that five syphilis patients (16.7%) tested positive for HIV [10].

A prior study by Anita Alawiah, found that 44 respondents (86.6%) were infected with syphilis. The PCR method for examining Treponema pallidum relies on selecting specific sequences as target genes from the Treponema pallidum genome. This study used PCR testing methods such as conventional PCR, reverse transcriptase PCR, nested PCR, multiplex PCR, and real-time PCR. Real-time PCR is the latest modification of conventional PCR methods. Its working principle is similar to conventional PCR, but it can quantify and track DNA amplification in real time. The instrument monitors DNA amplification as it occurs [11]. PCR operates by using a fluorescent reporter that binds to the target DNA and emits fluorescence signals, indicating the amount of product formed [12]. The PCR test for Treponema pallidum can use various clinical specimen types, depending on the disease's progression. The type of PCR method, the specimen used, and the target gene influence the specificity and sensitivity of molecular PCR results [11].

Research by Jacksen et al. (2024) emphasized the importance of detecting human papillomavirus (HPV) genotypes as a preventive measure in cervical cancer development [3]. Using Multiplex PCR and Flow Through Hybridization methods, this study successfully identified 35 HPV genotypes, including 18 high-risk genotypes and 17 low-risk genotypes. The results revealed a high prevalence of HPV-52 as a high-risk genotype and HPV-11 as a low-risk genotype frequently found in co-infections. These findings confirm that early detection of HPV plays a crucial role in preventing sexually transmitted infection-related cancers. The study also serves as a foundation for developing more comprehensive detection methods for other STIs, including tests capable of detecting multiple pathogens in a single test, as in this study, which detected eight bacteria, one protozoan, and two types of herpes viruses.

From the findings of these studies, it is clear that medical diagnosis of sexually transmitted infections still primarily uses real-time PCR methods, and there has been limited use of Multiplex PCR and hybridization techniques. This prompted our research team to explore these methods.

Advances in biomedical technology, particularly in molecular diagnostics, offer new possibilities for addressing these challenges. Technologies like multiplex PCR and hybridization allow simultaneous detection of multiple pathogens in a single sample, reducing the time and costs associated with STD testing. The HybriSpot 12 Manual platform, integrating the STD Direct Flow Chip, represents a significant advancement in diagnostic technology, enabling rapid and accurate detection of multiple pathogens with a single test.

This research aims to evaluate the effectiveness and efficiency of the HybriSpot 12 Manual platform in detecting sexually transmitted infections among diverse populations in the Jakarta. By utilizing the STD Direct Flow Chip, this study focuses on the simultaneous detection of 11 different pathogens, including bacteria, viruses, and protozoa responsible for various STDs. The study is designed to assess the prevalence of single infections and co-infections across various demographics, considering factors such as gender and age group.

The results of this study are expected to provide valuable insights into the distribution and dynamics of STDs in the Jakarta metropolitan area. Using the data obtained, the authors aim to improve understanding of STD transmission patterns and the effectiveness of current interventions. Additionally, this research will highlight the benefits of using advanced diagnostic technologies in public health, with potential implications for clinical decision-making and more targeted public health interventions.

2. Materials and Methods

2.1 Study Design

This study uses a cross-sectional observational study design conducted from January 2020 to December 2023. Cross-sectional studies are a type of observational research that collects data from a representative population or subset at a specific point in time [13]. This method allows researchers to collect comprehensive data on the prevalence of STDs and related factors in the target population, providing a solid basis for epidemiological analysis and planning of public health interventions. This study follows a systematic research process as illustrated in the following flow diagram:

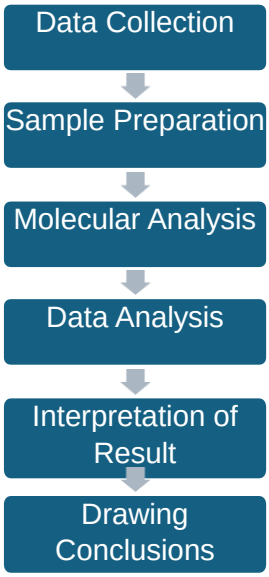

Fig.1. Research Process Flowchart

2.2 Population and Sample

This study focuses on individuals who undergo PMS screening at IMOQ LAB Jakarta during the period January 2020 to December 2023. This population was selected based on the consideration that IMOQ LAB is a health facility that provides STD screening services, thus offering a representative picture of the prevalence of STDs in the Jakarta area. This study uses the purposive sampling method, which is a non-probability sampling technique that allows sample selection based on population characteristics and research objectives [14]. This study applies several criteria to ensure the quality of the data obtained is accurate. The criteria include an age range between 17 and 82 years to represent the adult population that is sexually active to the elderly, willing to participate voluntarily, and not taking antibiotics in the last two weeks prior to sampling to avoid false negative results due to suppression of pathogen growth. Through these application criteria, this study succeeded in collecting 432 urine samples from participants with diverse work backgrounds. This sample size is considered to be able to provide accurate prevalence estimates and allow for stratified analysis based on the demographic characteristics of the participants.

2.3 Data Collection Techniques

The data in this study was collected through two main sources, namely primary data and secondary data. Primary data collection was carried out through biological sampling in the form of morning urine stored in sterile containers, where the selection of this collection time was based on previous research which showed that the sample had a higher concentration of pathogens [15]. Urine samples are stored at 2-8°C and processed within 24 hours to maintain specimen quality. In addition, primary data also includes demographic data collected through structured interviews using validated questionnaires. Meanwhile, secondary data was obtained from the results of laboratory analysis of urine samples using the HybriSpot 12 Manual platform, which included the detection of 11 pathogens related to PMS.

3. Results and Discussion

This study was conducted on 432 individuals aged between 17 and 86 years. Age was grouped in 10-year intervals, and the results showed 128 samples in the 17–26 age range (29.63%), 191 samples in the 27–36 age range (44.21%), 70 samples in the 37–46 age range (16.20%), 30 samples in the 47–56 age range (6.94%), 11 samples in the 57–66 age range (2.55%), 1 sample in the 67–76 age range (0.23%), and 1 sample in the 77–86 age range. Table 1 displays the total number and percentage of samples collected from the 17–86 age range.

Table 1. The number and total percentage of the sample range from ages 17 to 86 year

Age (Year)	Total	
	N	%
17 – 26	128	29,63
27 – 36	191	44,21
37 – 46	70	16,20

47 – 56	30	6,94
57 – 66	11	2,55
67 – 76	1	0,23
77 – 86	1	0,23

Among individuals suspected of suffering from sexually transmitted infections, sample examinations revealed a high infection rate exceeding 50%. Specifically, 255 out of a total of 432 samples tested positive for pathogens causing sexually transmitted diseases, yielding a percentage of 59.03%. This included 54.66% of positive cases (88 samples) from male patients and 45.34% (73 samples) from female patients. In cases of coinfection (where more than two pathogens causing sexually transmitted infections were found), the infection rate was 48.94% among male patients with 46 samples, and 51.06% among female patients with 48 samples. There were 173 negative samples, accounting for 40.05%, consisting of 120 male samples (69.36%) and 53 female samples (30.63%). Additionally, this study identified four invalid samples (0.93%) that were unreadable by the PCR machine, all from male patients. Two types of chips, negative and positive, are illustrated in Figure 2 and 3, while the differences in chip types for one pathogen and coinfection are shown in Figure 4 and 5.

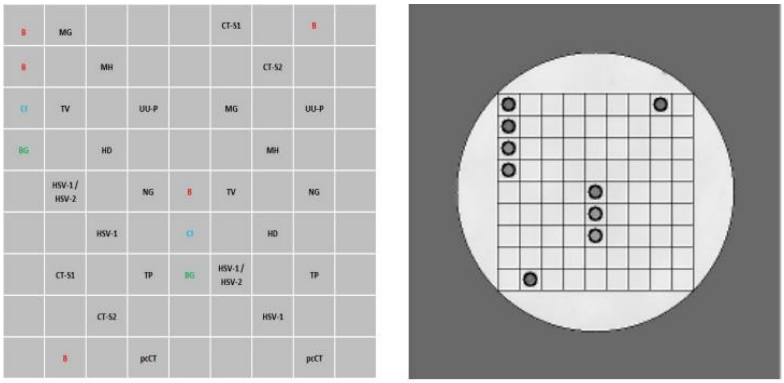

Fig. 2. Negative Chip

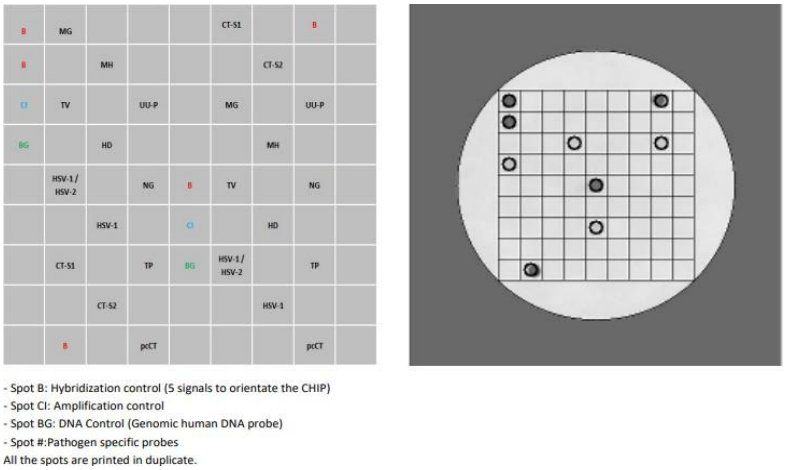

Fig. 3. Positive Chip

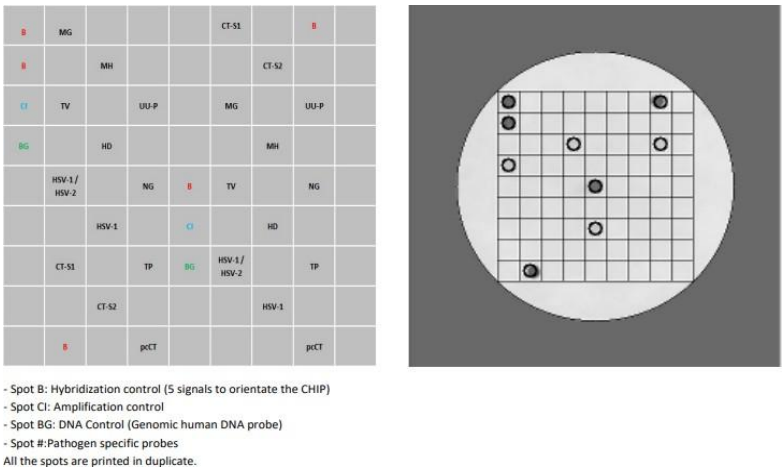

Fig. 4. One Pathogen Chip

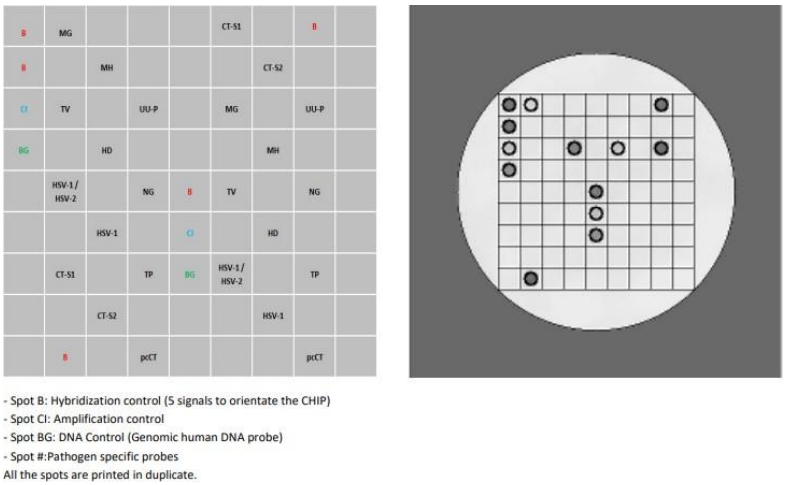

Fig. 5. Co-infection Chip

The majority of positive samples in this study contained only one type of pathogen, comprising 161 out of 255 positive samples (63.14%) from both male and female patients. In contrast, only 94 out of 255 cases (36.86%) exhibited positive samples with coinfection. Figure 6 presents the percentage of positive cases, and Table 2 outlines the total number and percentage of positive and negative cases for all samples tested in the study, as well as the number and percentage of samples infected with one or more pathogens capable of causing sexually transmitted infection.

Table 2. The total number and percentage of positive and negative cases for all samples tested

Infection		Total	
		N	%
Positive STD		255	59,03
a.	1 type of pathogen	161	63,14
	• Male	88	54,66
	• Female	73	45,34
b.	Co-infection	94	36,86
	• Male	46	48,93
	• Female	48	51,06
Negative STD		173	40,04
• Male		120	69,36
• Female		53	30,63
Invalid		4	0,93

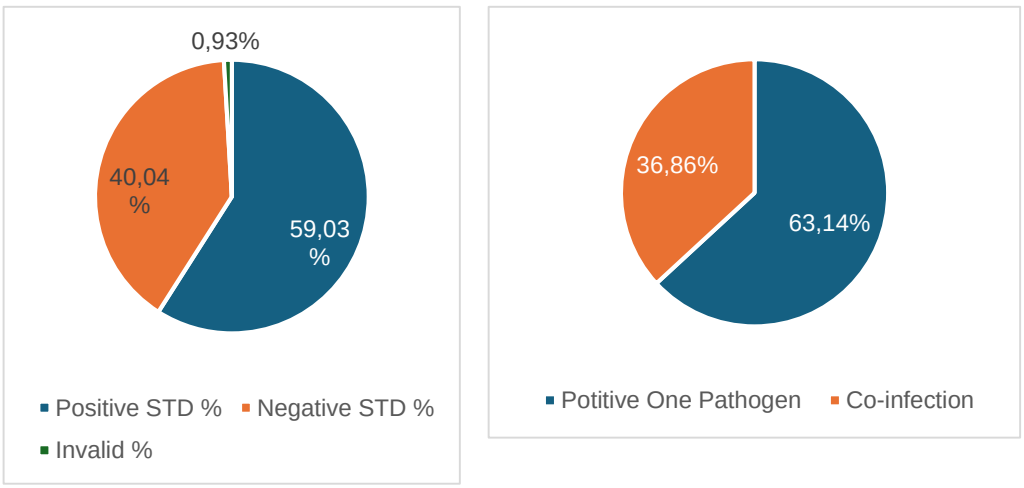

Fig. 6. Percentage differences between (a) positive, negative and invalid sources (b) positive 1 pathogen and co-infection

The DNA FLOW technique using the STD direct flow chip kit can identify various types of pathogens responsible for sexually transmitted infections (STIs). This method is characterized by rapid diagnosis and result interpretation in less than four hours, as well as its ability to analyze different clinical samples without the need for DNA extraction. The study's findings were obtained by first conducting a multiplex amplification process using a PCR mix tube, followed by insertion

into a thermal cycler program, and concluded with reverse hybridization [14,15]. The flow chip technique presents a potential alternative to standard diagnostic processes as it allows for direct sample examination and identification of a wide range of infections [14]. This research also identified 12 distinct pathogens associated with sexually transmitted infections, including *Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae, Trichomonas vaginalis, HPV simplex types 1 and 2, Chlamydia trachomatis (serovars A-K), Chlamydia trachomatis: A-L3, Haemophilus ducreyi, and Treponema pallidum.* Table 3 displays the quantity and percentage of each type of pathogen found. The highest percentage was observed for the *Ureaplasma*'s genus, both for *Ureaplasma urealyticum* and *Ureaplasma parvum*, in patients infected with only one pathogen, with a value of 68.32%, comprising 52 males (47.27%) and 58 females (52.73%). However, no patients infected with *Haemophilus ducrey*i and *Treponema pallidum* were identified.

Table 3. Number and Percentage of Pathogens Found

Infection	Total	
	N	**%**
U. urealyticum/parvum	110	68,32
• Male	52	47,27
• Female	58	52,73
M. genitalium	4	2,48
• Male	3	75
• Female	1	25
M. hominis	22	13,66
• Male	13	59,09
• Female	9	40,91
N. gonorrhoeae	7	4,35
• Male	6	85,71
• Female	1	14,29
T. vaginalis	1	0,62
• Male	0	0
• Female	1	100
HPV simplex type 1	2	1,24
• Male	2	100
• Female	0	0
HPV simplex type 2	4	2,48
• Male	3	75
• Female	1	25
Chlamydia trachomatis (serovares: A-K)	10	6,21
• Male	8	80
• Female	2	20
C. trachomatis: A-L3	1	0,62
• Male	1	100
• Female	0	0

Co-Infection with 2 Pathogens	76	80,85
• Male	39	51,32
• Female	37	48,68
Co-Infection with 3 Pathogens	14	14,89
• Male	6	42,86
• Female	8	57,14
Co-Infection with 4 Pathogens	4	4,26
• Male	1	25
• Female	3	75

Along with sexual behavior (both heterosexual and homosexual), not using condoms is a primary reason for the increase in STIs [16]. These results align with previous research Barrientos (2020) that studied the etiology of STIs using 94 sperm samples, yielding a positive sample ratio of 57.4% [14]. In contrast, a study by Wendt et al. (2019) employing multiplex real- time PCR on 67 different samples found a positive sample percentage of 38.8%, which is less than 50% of the total samples evaluated [17]. In recent years, the frequency of STIs has gradually increased worldwide, necessitating greater public emphasis on STIs within educational agendas set by governments [18].

In this study, the percentage of samples with single pathogen infections was 63.14%, while the percentage of samples with infections from more than one pathogen was 36.86%. This is consistent with the aforementioned study by Wendt et al [17]. The findings also corroborate research by Fife et al. (2017), indicating that the frequency of STIs has risen. *Ureaplasma urealyticum* is a common cause of non-gonococcal urethritis in men and cervicitis in women, frequently detected among STIs in men [19]. Early exposure to *U. urealyticum* induces a more significant inflammatory response than subsequent exposures due to previous exposure disrupting the immune response to subsequent infections [20]. Esen et al. (2017) noted that *U. urealyticum* can cause other pathogenic species, identifying pathogens similar to those detected in this study, such as *Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae,* and *Chlamydia trachomatis*, which infected patients with 2-3 simultaneous coinfections [21]. Additionally, their research indicated that the percentage of patients with *U. urealyticum* was 52.9%, the highest among cases in their study, which aligns with the findings here, where cases of *U. urealyticum/parvum* had the highest percentage at 68.32% [21]. Conversely, in a study by Kriesel et al. (2016) analyzing urine samples from both sexes, the infection rate of *U. urealyticum* was found to be 6.1%, a lower percentage compared to this research [22].

According to the World Health Organization (2008), *Chlamydia trachomatis* is the most common treatable sexually transmitted infection in Europe and the United States. *C. trachomatis* infections can affect the urethra, rectum, and throat, and are more prevalent in young sexually active men. Infections with *C. trachomatis* is also associated with increased risks of infertility and recurrent miscarriage [16,18,23,24]. Kularatne et al. (2018) found that 6% of patients had *C. trachomatis*, a figure that corresponds closely to the 6.21% found in this study [25]. In another study, Barrientos et al. (2020) evaluated eleven types of STIs using similar multiplex PCR technology on urine samples from individuals engaging in unrestricted sexual activities, reporting a *C. trachomatis* infection rate of 44.93%, the highest percentage among positive STI samples and differing from the percentage found in this study [14].

Moreover, this research also identified *Neisseria gonorrhoeae*, a pathogen responsible for infections transmitted through sexual contact. *Neisseria gonorrhoeae* infections typically affect the

urethra, endocervix, and rectum, and are believed to be the second most common sexually transmitted pathogen. The incidence of new gonorrhea cases is usually high in developing countries, where symptomatic men are more likely to seek treatment and preventive care to avoid complications. Regular screenings are recommended for sexually active individuals at higher risk [18,26]. In this study, cases of *Neisseria gonorrhoeae* were relatively low, at approximately 4.35%, consistent with Barrientos-Durán et al. (2020), who reported a similar percentage for *N. gonorrhoeae* infections [14].

In a study by Yokoi et al. (2007), the rate of co-infection with *Mycoplasma genitalium* among men with gonococcal urethritis was low, at 4.1%, compared to a co-infection rate of 21.2% with *C. Trachomatis* [27]. Other research indicated that among 45 men with gonococcal urethritis screened for *M. genitalium* using PCR, 4.4% tested positive [28]. In West Africa, Pepin et al. (2005) reported that nearly half of STI cases were caused *by M. genitalium*, often occurring as a co-infection [29]. This aligns with the current findings showing that co-infections involving 2-4 pathogens were largely due to the presence of *M. Genitalium*.

The use of STD Direct Flow Chips has enabled the simultaneous detection of several sexually transmitted disease (STD) pathogens in the region. A previous study by Sausen et al. (2023) reported that this technology has a sensitivity of 98.4% and a specificity of 99.9%, demonstrating its accuracy in detecting STD infections [30]. Our results show a prevalence of STD infections that is comparable to those found in other areas. The detection of co-infections with multiple pathogens in a number of patients emphasizes the importance of comprehensive screening in the management of STD. Co-infections can complicate diagnosis and treatment, and increase the risk of transmission and further complications. As stated by Sausen et al. (2023), interactions between certain STD pathogens, such as *Herpes Simplex Virus (HSV) and Human Papillomavirus (HPV*), can contribute to the development of more serious complications, including cervical cancer [30]. Therefore, early detection through reliable diagnostic methods is essential for long-term prevention. The implementation of the STD Direct Flow Chip in Jakarta has several advantages, including faster detection times and the ability to identify multiple pathogens in a single procedure. This is crucial in a clinical context to ensure that patients receive timely and appropriate therapy. In addition, this approach can contribute to controlling the spread of STDs in the community through early detection and effective treatment.

Despite its great potential, this technology still faces several challenges such as the need for adequate laboratory resources and training of health workers in the use of this technology. The presence of a possible false-negative result or false positive, which requires further confirmation with other diagnostic methods. As noted by Sausen et al. (2023), although PCR methods are highly sensitive, variables such as sample contamination, reagent quality, and genetic mutations in pathogens can affect the final result [30]. In addition, the study did not find any detection of *Treponema pallidum* in urine samples. Several factors may explain this limitation, including the location of the primary infection and the low concentration of pathogens. *Treponema pallidum* is mainly present in primary lesions or in the bloodstream during active syphilis infection, and is rarely found in the urinary tract, resulting in a very low or non-b chance of excretion in the urine. In addition, urine samples may contain only a small amount of *Treponema pallidum* because syphilis usually does not cause direct infection of the urinary tract. The Hybrispot 12 manual offers a wide selection of samples for the detection of PMS other than urine, such as Dacron swabs in sterile tubes and Liquid Based Cytology (LBC). The optimal approach to detecting *Treponema pallidum*, the causative agent of syphilis, may involve the use of swab samples from genital, urethral, or anal lesions, depending on the patient's history of STD as diagnosed by the Medical Agent.

However, overall, the application of STD Direct Flow Chip in the Jakarta area shows great potential in improving the detection and handling of STD. This rapid and accurate diagnostic approach could serve as a model for other regions in their efforts to combat the spread of sexually transmitted diseases. With better early detection, faster treatment, and a deeper understanding of co-infection patterns, this approach can help reduce the public health burden of STIs.

4. Conclusion

This study successfully revealed the prevalence of sexually transmitted infection (STI) pathogens using the Hybrispot 12 Manual - STD Direct Flow Chip technology on 432 samples from the Jakarta Special Region. The results indicated that over half of the samples tested positive for STI pathogens within the age range of 17 to 89 years, with the majority of cases involving single infections (63.14%) and the remainder consisting of coinfections (36.86%). In cases of coinfection, most infections were attributed to *Mycoplasma genitalium/hominis*. The most prevalent pathogens causing sexually transmitted infections were *Ureaplasma urealyticum, Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae,* and *Chlamydia trachomatis*. The multiplex PCR-based diagnostic technology on the HybriSpot 12 Manual platform proved to be accurate and efficient in simultaneously detecting a variety of pathogens, thereby supporting quicker and more accurate clinical decision-making. However, the detection of *Treponema pallidum* was somewhat challenging due to the use of urine samples from patients in this study.

Based on the findings of this study, continuous education efforts are needed to inform the public about the importance of prevention and routine screening by related parties. Health campaigns that focus on safe sexual practices are expected to help curb the spread of STIs. Seeing the advantages of the accuracy and speed of Hybrispot 12 Manual - STD Direct Flow Chip technology, it is recommended that this technology be used more widely in other health facilities, especially in areas with high PMS rates, to speed up the diagnosis and treatment process. In addition, collaboration with government entities and non-governmental organizations (NGOs) is highly recommended to strengthen public health intervention efforts aimed at reducing the prevalence of STIs.

5. Acknowledgment

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