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Economic implications of ddPCR and NGS-based noninvasive prenatal testing for fetal aneuploidy screening

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ABSTRACT

Noninvasive prenatal testing (NIPT) heralds a transformative era in prenatal care, revolutionizing fetal health assessment. The recent adoption of aneuploidy screening signifies a significant advancement in prenatal genetic care in India. The current study aimed to assess the cost-effectiveness of ddPCR-based NIPT for detecting chromosome aneuploidies, comparing it with the next-generation sequencing (NGS) platform. This study adopts a laboratory-based observational approach to investigate the cost implications of NIPT for trisomies 13, 18, and 21 using ddPCR and NGS technologies. A meticulously designed cost analysis methodology was employed, adhering to established standards. The yearly capital and operational costs of NIPT were calculated with precision, focusing on the specific methods associated with ddPCR and NGS. The calculated annual capital and operating costs for NIPT using the ddPCR were $16,411 and $246,540 while those using the NGS platform were $91,440 and $250,560, respectively. The total cost of NIPT using ddPCR for 2,400 tests was $262,951, with an estimated cost per test of $110. In contrast, the total cost of NIPT using the NGS platform for 600 tests was $342,000, resulting in an estimated cost per test of $570. The ddPCR is five times more cost-effective. Moreover, it exhibits a fourfold reduction in time expenditure, attributable to streamlined procedures and does not require a complex bioinformatics analysis compared to the NGS. Moreover, this preliminary outcome on cost analysis for NIPT using ddPCR, as opposed to the NGS platform, can be extended to the health technology assessment (HTA) perspective for prenatal screening programs.

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1. INTRODUCTION

Prenatal testing constitutes a crucial and proactive strategy in averting the birth of offspring affected by genetic disorders [1]. These testing modalities encompass both non-invasive and invasive procedures. The accurate identification of chromosomal aneuploidies during early pregnancy holds paramount significance for guiding pregnancy management and facilitating genetic counseling [2]. Non-invasive prenatal testing (NIPT) involves the examination of fetal deoxyribonucleic acid (DNA) present in maternal blood samples for diagnostic purposes. Among the prominent methodologies employed for NIPT, next-generation sequencing (NGS), chromosomal microarray (CMA), and droplet digital PCR (ddPCR) stand out as the widely adopted approaches. NGS-based NIPT, in particular, is frequently utilized to detect prevalent fetal genetic aneuploidies [3]–[5]. Nevertheless, it is pertinent to acknowledge that a considerable cost per test accompanies this method.

Over the last decade, technological advancements in advanced molecular methodologies have significantly reduced the cost of DNA-based diagnosis, making diagnostic testing more accessible. A ddPCR, an emerging approach for detecting chromosomal aneuploidies, has garnered increasing acclaim owing to its operational efficiency. Notably, ddPCR demonstrates temporal expediency and obviates the necessity for specialized bioinformatics tools for data analysis. On the other hand, NGS-based testing methods involve intricate procedures, high cost, time-intensive processes, and require high-resource intensive settings [4]–[7]. To enhance the clinical effectiveness of NIPT for widespread prenatal screening, a multiplexed ddPCR-based assay can harness cell-free fetal DNA (cffDNA) to identify fetal trisomies 13, 18, and 21 in a single reaction [8]–[11]. However, cffDNA is present at extremely low concentrations within maternal DNA. ddPCR is an advanced technique that divides a 20 µL polymerase chain reaction (PCR) reaction into 20,000 droplets, significantly enhancing the test's sensitivity and specificity by several orders of magnitude. Various studies have emphasized that ddPCR is the most robust method for precisely quantifying minute amounts of DNA and can potentially develop a compassionate and reproducible NIPT method [12].

Furthermore, ddPCR-based NIPT has significant potential for being more upfront, rapid, and cost-effective than NGS-based NIPT; a considerable challenge arises from the necessity of many PCR-positive reactions to ensure clinical reliability [11]–[14]. This is primarily due to the low fraction of cell-free fetal DNA in maternal plasma. The NGS-based NIPT test is a widely established method for screening fetal genetic aneuploidies via DNA sequencing. In contrast, the application of ddPCR-based NIPT for fetal aneuploidy detection has received limited attention in the literature. Nevertheless, ddPCR shows promise as a prenatal screening option due to its potential for enhanced effectiveness, accessibility, convenience, cost-effectiveness, and time-saving compared to NGS [15].

Considering these impressive attributes of ddPCR, the current study conducted a cost analysis to determine the cost per test for ddPCR versus NGS-based NIPT. This article aims to assess the cost analysis of NIPT to identify fetal aneuploidies using ddPCR in comparison with NGS. Here, we calculated the capital cost and operational cost of the NIPT test to detect fetal aneuploidy (trisomies 13, 18, and 21) using ddPCR versus NGS-based NIPT assay for clinical utility, challenges, and advantages.

2. METHOD

All financial data was collected from the ongoing test facility at the All-India Institute of Medical Sciences in Jodhpur, India, from December 2021 to June 2022. We employed the standard cost calculation methodology to determine the cost per test for NIPT using both NGS and ddPCR techniques. The investigation encompassed both capital and operating cost analysis for both methods and a detailed breakdown of expenses is outlined in Table 1.

Table 1. A description of costs, categorized by specific heads, is provided for establishing an NIPT lab

S. No.	Cost as per heads	Description
1.	Capital assets cost	Building Costa (genetic laboratory dedicated to NIPT testing facility)
		Equipment (NGS, ddPCR, QC tap station, DNA isolation machine, and other small equipment)
		Furniture & fixtures
2.	Operating cost	Human resources for instrumentation operation and results in interpretation (scientist and lab technician
3.	Consumables	Kits for test procedures, plastic, and glassware
		Sample collection and processing, and report printing
4.	Other costs	Instrument maintenance cost
		Electricity cost

Building costs included the estimated monthly rent of the laboratory hall

2.1. The annualized cost of capital assets

The annual cost of capital assets was determined by dividing the procurement cost of machines by their average lifespan, assumed to be five years in this study [16], [17]. Equipment costs obtained from the institute's finance department include small instruments required for NIPT, such as deep freezers, regular freezers, tables, chairs, and air conditioners. Building costs were estimated based on monthly laboratory space rent. Detailed costs and the cost analysis are presented in supplementary in appendix.

2.2.1. Operating cost

The operating cost included a monthly salary of dedicated scientific and technical staff to run the lab and equipment. The laboratory staff's responsibilities included sample collection, DNA isolation, quantification, PCR reaction preparation, instrument operation, and data analysis. The salaries of technical and scientific persons were taken as per government standard norms. The NIPT test is a prerequisite for downstream applications involving cell-free DNA. Additionally, determining the fetal fraction is a pivotal and intricate procedure that demands expertise for its execution. Moreover, the NGS data analysis necessitates a dedicated bioinformatician's involvement.

The operating cost comprises consumables related to capital items, including chemicals, kits, reagents, and electricity. Data on these costs were gathered from various suppliers and vendors over six months, aligning with the instrument requirements for NIPT testing (ddPCR and NGS). The monthly electricity consumption cost was derived by calculating the daily power consumption and multiplying it by the unit cost. This comprehensive approach ensures a detailed understanding of operational expenses.

2.2.2. Test throughput

The NIPT methods include both ddPCR and NGS-based tests for detecting fetal aneuploidy. This study estimated the capacity of a single setting to perform approximately 200 tests using ddPCR and 50 tests using NGS monthly. The cost analysis for NIPT via ddPCR and NGS covers the 2021-2022 financial year. The anticipated lifespan for instruments, small instruments, furniture, and other fixtures is assumed to be five years, with 100% depreciation. NIPT per test cost (NGS/ddPCR) was derived using the formula:

$$\frac{\text{Total capital cost+Total operating cost}}{\text{Total test of NIPT conducted}} \quad (1)$$

3. RESULTS AND DISCUSSION

Next-generation sequencing enables in-depth, high-throughput exploration of molecular mechanisms. Mutation analysis using the NGS platform has become increasingly predominant in recent times. While the test cost and run-around time have significantly decreased, the expense associated with complex test procedures remains challenging for the NGS platform. In this study, we emphasize a cost-effective diagnostic approach for chromosome aneuploidy using ddPCR, examining the associated costs from a health economics perspective. While limited research has focused on the cost analysis of NIPT testing for chromosome aneuploidy, selective studies from various regions consistently underscore the lower cost of NIPT with ddPCR compared to NGS [18]–[25]. Despite these findings, a comprehensive and detailed cost analysis study remains necessary.

The ddPCR platform for NIPT has excellent potential to be more forthright, prompt, and cost-effective than NGS [26]. The ddPCR approach is highly sensitive and specific, with a minimal risk of false-positive results. The ddPCR can determine a low fraction of cell-free fetal DNA in maternal blood and amplify each target and reference amplicon [18]. Also, ddPCR-based NIPT requires a lower cost of equipment and reagents, which decreases the detection cost compared to NGS [27]. Multiplexed ddPCR-based NIPT testing is convenient and cost-effective, encouraging ddPCR-based NIPT as a competitive prenatal testing method for clinical use [19]. The multiplexed ddPCR NIPT assay can detect aneuploidies (trisomies 13, 18, and 21) in a single tube reaction with a similar level of sensitivities and specificity to NGS and is cost-effective [28]. NIPT through NGS requires an advanced molecular laboratory setup, well-trained human resources, and high-cost equipment investments. In addition, an advanced bioinformatics setup is also required. These specifications collectively contribute to the overall high cost per test. NGS testing is exclusively viable in centralized laboratories, ensuring streamlined sample procedures and expediting data analysis [8].

3.1. NIPT cost analysis through ddPCR

The calculated annual capital and operational cost of ddPCR-based NIPT tests are detailed in Table 2. The building and maintenance costs for the ddPCR facility for the current year were estimated at INR 1,34,000. The total annualized cost of ddPCR NIPT test facility equipment was estimated to be INR 10,00,000. Maintenance costs were not separately included, as they were already accounted for within the equipment cost, covering comprehensive maintenance for five years. The cost of small instruments necessary for laboratory operations was determined to be INR 80,000 annually. The annual cost for furniture and other fixtures was found to be INR 50,000. The human resource cost for the ddPCR facility was estimated at INR 12,60,000 per year. The yearly cost of consumables, test kits, and reagents/chemicals for conducting 2,400 tests was INR 1,75,84,800. The shared annual electricity cost for the ddPCR facility was INR 1,20,000. The following nature of NIPT utilizing ddPCR aligns with findings from previously reported studies by Wang *et al.* [29].

3.2. NIPT cost analysis through NGS

In the context of NIPT, the NGS test incurred laboratory room and maintenance costs totaling INR 1,34,000 for the current year. The overall annualized expenditure for NGS facility equipment reached INR 60,00,000, with equipment maintenance costs embedded, covering comprehensive maintenance for five years. Operating the laboratory with essential small instruments amounted to an annual cost of INR 4,00,000, while furniture and fixtures incurred a yearly expense of INR 5,00,000. Human resource costs for the NGS facility were estimated at INR 21,00,000 per year. The annual expenses for consumables, test kits, and reagents/chemicals to conduct 600 tests were INR 1,68,18,000. The yearly electricity cost amounted to INR 3,60,000. Detailed price breakdowns can be found in Table 3 for NIPT via NGS. Comparatively, the capital and operating costs for NIPT via ddPCR were INR 70,34,000 and INR 1,92,78,000, while for NIPT via NGS, they were INR 12,64,400 and INR 1,89,64,800, respectively. The elevated expense associated with NIPT using NGS concurs with findings from prior reported studies by Xiao *et al.* [30]. Further cost details are available in the NIPT supplementary in appendix for ddPCR and NGS.

Table 2. Annual cost for NIPT via the ddPCR method (test calculation was performed on a monthly and yearly basis; detailed information is available in supplementary in appendix)

S. No.	Type of cost	Monthly (INR)	Annually (INR)
1.	Capital cost (INR)		
	Building rent along with maintenance	11,167	1,34,000
	Equipment	83,333	10,00,000
	Other small equipment	6,667	80,000
	Furniture and other fixtures	4,167	50,000
	Total	1,05,334 ($1,368)	12,64,400 ($16,411)
2.	Operating cost (INR)		
	Manpower	1,05,000	12,60,000
	Consumables kits, reagents, etc.	14,65,400	1,75,84,800
	Electricity	10,000	1,20,000
	Total	15,80,400 ($20,545)	1,89,64,800 ($246,540)
	Total (1+2) (INR)	16,85,734 ($21,913)	2,02,29,200 ($2,62,951)
	Test conducted	200	2400
	Per test cost	Approx. INR 8,430 ($110)	

Table 3. Annual cost for NIPT via NGS method (test calculation was performed on a monthly and yearly basis; detailed breakup information available in supplementary in appendix)

S. No.	Type of cost	Monthly (INR)	Annually (INR)
1.	Capital cost (INR)		
	Building rent along with maintenance	11,167	1,34,000
	Equipment	5,00,000	60,00,000
	Other small equipment	33,333	4,00,000
	Furniture and other fixtures	41,667	5,00,000
	Total	5,86,167 ($7,620)	70,34,000 ($91,440)
2.	Operating cost (INR)		
	Manpower	1,75,000	21,00,000
	Consumables kits and reagents	14,01,500	1,68,18,000
	Electricity	30,000	3,60,000
	Total	16,06,500 ($20,880)	1,92,78,000 ($2,50,560)
	Total (1+2) (INR)	21,92,667 ($28,500)	2,63,12,200 ($3,42,000)
	Test conducted	50	600
	Per test cost	Approx. INR 43,850 ($570)	

The costs associated with NIPT through ddPCR and NGS may decrease with an increase in the number of tests performed. Evidence on the cost of NIPT tests in low and middle-income countries (LMICs) is currently unavailable. However, in governmental and private healthcare settings, charges range from approximately $500 to $2,000 per test [31], [32]. Prices vary globally, influenced by infrastructure and laboratory facilities at the country level. NIPT costs per test range from $795 to over $3,000 in the USA. European prices range from €631 to €858, and in the United Kingdom, from £400 to £900 [32]. In Hong Kong, the cost is approximately HK$4,500 to 8,000 ($580 to $1,000), and in Brazil, it is R$3,500 ($1492) [32]. Though still considered high, Dai *et al.* in 2022 reported that NGS and microarray-based NIPT tests are $100 to $200 [26]. Notably, health insurance typically does not cover this test, placing the entire cost burden on the patient and increasing out-of-pocket expenditure.

Table 2 and 3 describe a comprehensive breakdown of the capital and operating cost of the NIPT through ddPCR/NGS test services. Conducting 2,400 tests annually, the ddPCR NIPT test incurs an annual cost of INR 2,02,29,200 ($264,000), with 7% attributed to capital costs and 93% to operating costs, see Figure 1. In contrast, the annual cost for NIPT through NGS services is INR 2,63,12,200 ($342,000) for 600 tests, with 27% allocated to capital costs and 73% to operating costs, see Figure 2. The ddPCR NIPT testing facility expenditure primarily comprises three significant components, contributing to over 90% of the total cost. On the other hand, NGS facility expenditure is mainly comprised of three critical elements, contributing to more than 75% of the cost, namely equipment cost, workforce cost, and the cost of consumables, see Figure 2. Examining the unit costs, the annual capacity for NIPT tests via ddPCR is 2,400, resulting in a unit cost of INR 8,430 ($110). Conversely, for NIPT tests via NGS with an annual capacity of 600, the unit cost is INR 43,850 ($570). The utilization of NGS for NIPT entails a larger laboratory footprint, an increased requirement for furniture and fixtures, additional manpower for data analysis, and higher electricity consumption, contributing to the observed cost variations. This information is summarized in Figures 1 and 2. The cost-effectiveness of NIPT using ddPCR and the elevated cost of NIPT using NGS are in line with conclusions from studies reported earlier by Wang *et al.* and Xiao *et al.* [29], [30].

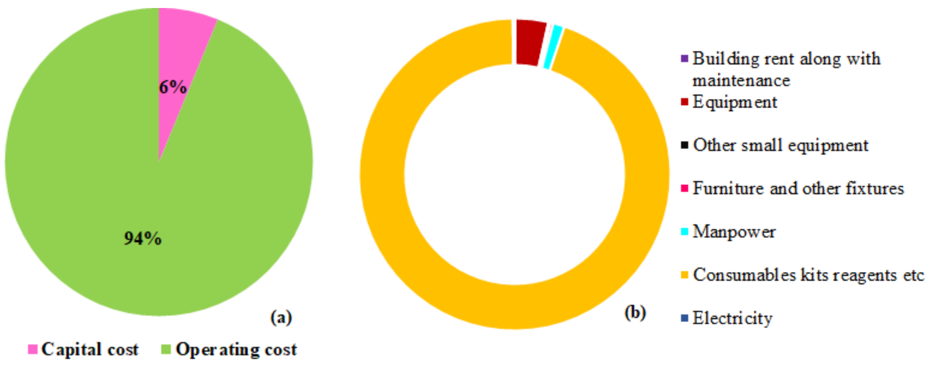

Figure 1. Cost of NIPT via ddPCR, (a) Proportion of operating and capital cost, (b) Cost breakdown

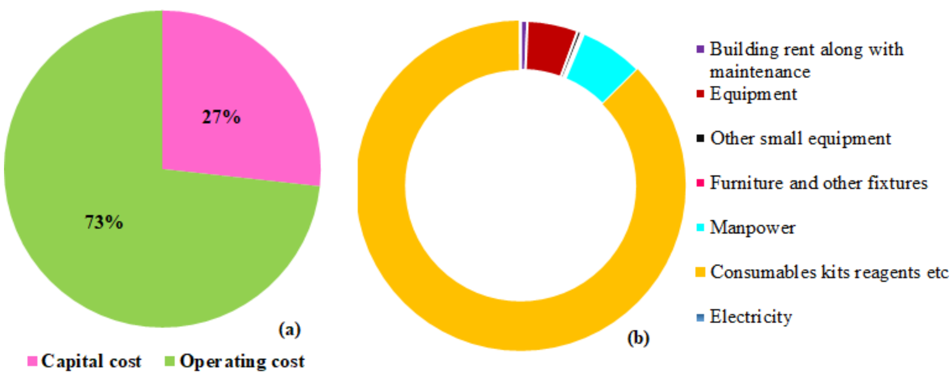

Figure 2. Cost of NIPT via NGS, (a) Proportion of operating and capital cost, (b) Cost breakdown

Over the past five years, sequencing technology advancements have led to the emergence of various output sequencing platforms, including single molecule, real-time (SMRT) sequencing by PacBio, Nanopore by Oxford Nanopore Technologies, Genia nanopore, and others. These platforms boast the capability to read over 5,000 bases per run. However, despite their high throughput, the test procedures are time-consuming, requiring three days for a single read, and have a significant cost [33].

In India, the landscape of NIPT testing laboratories is limited, with the majority adopting the NGS platform for aneuploidy detection by Verma *et al.* [34]. The standard cost of NIPT in India currently hovers around Rs 25,000. A stark contrast from half a decade ago when the cost per test ranged from Rs 50,000 to Rs 60,000 ($700 to $1000). Most testing was outsourced to developed countries such as the USA, Europe, and the UK during that period. However, in recent years, a select few scientific laboratories have initiated testing within the country, contributing to a reduction in the cost of NGS testing. Despite this positive trend, the availability of NIPT facilities remains concentrated in private research centers in metro cities in India, and the overall number of laboratories is still limited.

A cost-cutting technique can be applied to reduce NIPT's capital and operating costs, ultimately decreasing the per-test cost. Regarding capital costs, adopting the ddPCR platform for NIPT can be an attractive alternative to NGS from a government perspective. This transition can potentially curtail additional equipment, furniture, and consumables expenses. Operational cost reduction strategies may include establishing district-level testing centers for batch processing, optimizing employee utilization, implementing a well-structured protocol, and incorporating parallel testing. Considering a potential price drop and the ongoing expansion of NIPT to include chromosome abnormalities beyond T21, T18, T13, and sex chromosome aneuploidies, future research should examine the potential cost-effectiveness of implementing NIPT as the first-line test [35].

4. CONCLUSION

The current study has shown the alternative of the NGS platform to determine chromosomal aneuploidy using NIPT. ddPCR is a sensitive and robust technique to ascertain the mutation using minute, even very low DNA concentrations. Additionally, ddPCR is a cost-effective method for NIPT screening, costing $110 per test compared to NGS, which costs $570 per test. ddPCR is five times more cost-effective than the NGS-based test. ddPCR offers a more straightforward test procedure than NGS and does not necessitate intricate bioinformatic analysis. The ddPCR platform emerges as a favorable choice for prenatal screening, exhibiting strengths in effectiveness, accessibility, convenience, cost-effectiveness, and time-saving. The cost analysis methodology used in this study can also be used to evaluate cost benefits for various laboratory investigations. Moreover, this preliminary cost estimation outcome for NIPT testing using ddPCR compared to the NGS platform can serve as a foundation for a broader health technology assessment perspective in the context of prenatal screening programs.

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APPENDIX

Supplementary File: ddPCR NIPT per test cost calculation (INR)

(i) Fixed cost				
			Description of calculated instrument cost	
		Instrument	**Lifespan**	**cost**
		ddPCR	Approx life	
Building rent	10000		5 years	5000000
Tax	1200		for 1 month	**83333.33333**
Instruments cost/month		Cell-free DNA Isolation machine	Approx life	
ddPCR	**83333.33333**		5 Years	500000
Cell-free DNA Isolation machine	**8333.333333**		for 1 month	**8333.333333**
Other small instruments & furniture fixtures	**10833.33333**	AMC included for 5 years in cost		
		The total cost of other small instruments	650000	**10833.33333**
		Description of calculated salary of Technical staff		
Technical staff salary	**105000**	**Post**		Amount
		Technician		35000
		Research Fellow		70000
Electricity	10000	Total		**105000**
Total cost/month	**228700**			
Test sample load/month expected	**200**			
Fixed cost/sample	**1143.5**			

Supplementary File: ddPCR NIPT per test cost calculation (INR)

	(ii) Variable cost			
a. Indirect cost	Sample collection (EDTA tube, Syringe, Alcohol swab, and bandages)	15		
	Report printing	2		
	Storage and processing	10		
		27		
			for 50 tests	**Cost Per test**
b. Direct cost	1. Cell-free DNA isolation kit		70000	**1400**
	2. Fetal fraction detection kit		70000	**1400**
	3. NIPT kit ddPCR		205000	**4100**
	4. Other consumables (96 well plates cartridges gas kit aluminum foil tips etc.)		20000	400
				7300
Total cost per sample [(i) Fixed cost + (ii) Variable cost (indirect cost + direct cost)			**8470.5 INR**	

Supplementary File: NGS NIPT per test cost calculation (INR)

(i) Fixed cost		Description of calculated instrument cost		
		Instrument	**Life span**	**cost**
		MiSeq	Approx life	
Building rent	10000		5 years	30000000
Tax	1200		for 1 month	**500000**
Instruments cost/month		Cell-free DNA Isolation	Approx life	
MiSeq	500000		5 Years	500000
Cell-free DNA Isolation machine	8333.333333		for 1 month	**8333.333333**
Other small instruments furniture & fixtures	66666.66667			4000000
		The total cost of QC Tap Station and other small instruments and AMC included for 5 years in cost	Approx life 5 yearsv for 1 month	**66666.66667**
		Description of calculated salary of technical staff		
Technical staff salary	175000	**Post**		**Amount**
		Technician		35000
		Research Fellow Scientist (Molecular biologist + Bioinformatician)		140000
Electricity	30000	Total		**175000**
Total cost/month	791200			
Test sample load/month expected	50			
Fixed cost/sample	**15824**			

Supplementary File: NGS NIPT per test cost calculation (INR)

	(ii)Variable cost			
a. Indirect cost				
	Sample collection (EDTA tubes, Syringe, Alcohol swab, and bandages)	15		
	Report printing	5		
	Storage and processing	10		
		30		
			for 50 tests	**Per test cost**
b. Direct cost reagents	1. Cell-free DNA isolation kit		70000	1400
	2. Fetal fraction detection kit		70000	1400
	3. NIPT kit NGS (Flow cell Index, Adaptor, Probe, Primer, Master mix etc.)		1250000	25000
	4. Other consumables (96 well plates cartridges gas kit, aluminum foil, and other plastic wares)		10000	200
				28000
Total cost per sample (i) Fixed cost + (ii) Variable cost (indirect cost + direct cost)				**43,854 INR**

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