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COMPARATIVE EFFICACY OF HUMAN SERUM, HEPARIN PLASMA, AND EDTA PLASMA AS INDUCTION MEDIA FOR GERM TUBE FORMATION IN CANDIDA ALBICANS: IMPLICATIONS FOR ROUTINE DIAGNOSTIC MICROBIOLOGY

Clinical Microbiology

Mrs. Liliya Jacob* Associate Professor, Department of MLT St. Thomas College of Allied Health Sciences, Chethipuzha, Changanassery *Corresponding Author

Mrs Amalu Sabu Final Year Bsc. MLT Kerala University of Health Sciences, Thrissur, Kerala, India

ABSTRACT

Background: Candida albicans is a leading cause of opportunistic fungal infections, ranging from mucocutaneous disease to life-threatening invasive candidiasis. The germ tube test is a simple, rapid, and widely used presumptive method for identifying C. albicans, traditionally performed using human serum. However, serum preparation is time-consuming, expensive, and associated with biosafety risks from blood-borne pathogen exposure.**Aim:** To compare the efficiency of human plasma (heparin-anticoagulated and EDTA-anticoagulated) with human serum as induction media for germ tube formation in Candida albicans.**Materials and Methods:** A standardized C. albicans inoculum (ATCC 10231; 5 × 106 CFU/mL) was incubated with serum, heparin plasma, and EDTA plasma at 37 °C. Germ tube formation was examined microscopically at 60 and 120 minutes (n = 30 samples). Germ tube lengths were measured, and patient biochemical (glucose, cholesterol, total protein) and hematological (CBC, Hb, ESR) parameters were documented to evaluate their influence on germ tube induction. Statistical analysis was performed using the Friedman test (three-group comparison) and a paired t-test (serum vs. heparin plasma).**Results:** Candida albicans produced germ tubes in all three media with no false-negative results in serum or heparin plasma. Serum produced germ tubes of 15–20 µm at 60 minutes and 40 µm at 120 minutes. Heparin plasma showed comparable lengths (10–15 µm at 60 min; 25–40 µm at 120 min). EDTA plasma demonstrated markedly suppressed induction (0–15 µm at 60 min; 0–25 µm at 120 min). Friedman test revealed a highly significant difference among the three media at both time points ($p < 0.001$). A paired t-test comparing serum and heparin plasma at 120 minutes showed no statistically significant difference ($t = 0.00$; $p = 1.00$), confirming equivalent performance. Patient biochemical and hematological parameters showed no significant correlation with germ tube formation.**Conclusion:** Serum remains the most efficient medium for germ tube testing. Heparin plasma is a practical, safe, and statistically equivalent alternative to serum, offering reduced biosafety risks, lower cost, and improved laboratory workflow. EDTA plasma is unsuitable due to its inhibitory chelating effect on essential divalent cations required for germ tube induction. Heparinized plasma is recommended as a reliable substitute for serum in routine clinical microbiology laboratories.

KEYWORDS

Candida Albicans; Candidiasis; Germ Tube Test; Heparin Plasma; EDTA Plasma; Invasive Candidemia; Antifungal Diagnosis; Hyphal Formation

INTRODUCTION

Candidiasis, an infection caused by *Candida* yeast, particularly *Candida albicans*, is a significant cause of morbidity and mortality worldwide, affecting the nails, skin, oral mucosa, and in severe cases, the bloodstream and internal organs [1]. *C. albicans* is a ubiquitous, dimorphic yeast that typically resides as a commensal in the oral cavity but can become pathogenic under specific circumstances, causing oral thrush, mucocutaneous infections, vulvovaginitis, and life-threatening systemic invasive candidemia, especially in immunocompromised patients including those with AIDS, autoimmune diseases, and transplant recipients [2, 3].

Invasive candidiasis accounts for 15–30% of all nosocomial infections in critically ill patients [4] and represents nearly a third of nosocomial infections in intensive care units (ICUs) [5, 6]. Invasive mycosis is considered the second most frequent nosocomial infection in Peru and the fourth in the United States [7]. According to the WHO, an estimated 750,000 cases of invasive candidiasis occur globally each year, leading to 250,000–400,000 deaths annually [1].

Management of severe *Candida* infections is hampered by the lack of rapid and reliable diagnostic methods, which delay appropriate antifungal therapy [8]. Among available diagnostic tools, the germ tube test stands out as a cost-effective, rapid, and simple presumptive test for identifying *C. albicans* within 2–3 hours. Traditionally, fresh human serum serves as the induction medium; however, its preparation is time-consuming, costly, and carries biosafety risks associated with blood-borne pathogen exposure. This study evaluates human plasma — both heparin-anticoagulated and EDTA-anticoagulated — as safer and more accessible alternatives to serum for routine germ tube testing.

REVIEW OF LITERATURE

Candidiasis has emerged as one of the most prevalent opportunistic fungal infections in hospital and community settings, predominantly affecting immunocompromised patients. Among the diagnostic methods, the germ tube test is widely recognized as a simple, reliable, and presumptive test for *C. albicans* identification, yielding results within 2–3 hours and requiring only basic laboratory facilities [10].

Traditionally, fresh human serum serves as the inducing medium for germ tube formation, owing to its specific nutrients and proteins that stimulate hyphal outgrowth in *C. albicans*. However, serum handling is time-consuming, expensive, and associated with biosafety risks such as exposure to blood-borne pathogens. Human plasma, obtainable more easily from anticoagulated blood, has been proposed as a safer and more accessible alternative.

Hidayati et al. (2023) demonstrated that CPDA plasma exhibited a germ tube formation time close to serum (average: 70 min for serum, 129 min for CPDA plasma, 146 min for citrate plasma, and 162 min for EDTA plasma), confirming that EDTA plasma requires the longest time for germ tube induction [11]. UK Standards for Microbiology Investigations (SMI) recommend that germ tube assessment be completed within 2–3 hours, a window achievable with both serum and plasma alternatives [12].

Jeel Moya-Salazar and Roberto Rojas compared the germ tube test in human plasma with serum for identifying *Candida* species and reported excellent diagnostic agreement [6]. Mulyati et al. (2019) also explored egg white media, achieving germ tube positivity in 96.4% of isolates within 2–3 hours, though routine availability remains a concern [13]. Jan and Bashir demonstrated that the germ tube test performs comparably to PCR-based phenotypic methods, with significant advantages in cost and turnaround time [15]. These findings collectively support the exploration of plasma as a practical diagnostic alternative.

AIM AND OBJECTIVES

Aim

To compare the efficiency of human plasma (heparin and EDTA anticoagulated) with human serum as induction media for germ tube formation in *Candida albicans* clinical isolates.

Objectives

1. To evaluate and compare germ tube formation time and length at 60 and 120 minutes in serum, heparin plasma, and EDTA plasma by light microscopy.

2. To assess heparin plasma and EDTA plasma as safer and more readily available alternatives to serum for routine microbiology laboratories.

3. To determine the influence of patient biochemical parameters (blood glucose, cholesterol, total protein) and hematological profiles (CBC, Hb, ESR) on germ tube induction.

4. To recommend the most suitable alternative medium for routine clinical microbiology practice based on statistical evidence.

MATERIALS AND METHODS

Study Design and Ethical Considerations

This study was conducted as a prospective comparative laboratory analysis. Ethical approval was obtained from the Institutional Ethics Committee of St. Thomas College of Allied Health Sciences, Changanassery. Informed consent was secured from all participants for blood sample collection. All experiments were performed at the Department of Microbiology, BSc. MLT.

Inclusion and Exclusion Criteria

Inclusion Criteria:

- Fresh blood samples processed within 2–4 hours of collection.
- Clinical isolates of Candida albicans confirmed by microscopy and biochemical identification.
- Isolates showing viable growth and suitable for germ tube induction.

Exclusion Criteria:

- Non-albicans Candida species.
- Haemolyzed, lipaemic, or icteric blood samples.
- Improperly collected or old samples (>6 hours).
- Patients on antifungal therapy.
- Samples with bacterial contamination.

Sample Collection and Preparation

Blood samples were collected from 30 patients at St. Thomas Hospital, Chethipuzha. For serum preparation, blood was collected in plain (red-top) tubes, allowed to clot at room temperature, and centrifuged at 2500 rpm for 10 minutes. For plasma preparation, blood was collected in EDTA tubes (purple-top) and heparin tubes (green-top), immediately centrifuged at 2500 rpm for 10 minutes, and the supernatant plasma was separated aseptically. All samples were processed within 2–4 hours of collection.

Candida albicans Inoculum Preparation

Candida albicans (ATCC 10231) was cultured on Sabouraud Dextrose Agar (SDA) at 37 °C for 24 hours. A suspension was prepared in sterile normal saline to achieve turbidity equivalent to 0.5 McFarland standard (approximately 5×10^6 CFU/mL). *Candida glabrata* was used as a non-albicans negative control.

Germ Tube Induction and Microscopic Examination

One milliliter of each medium (serum, heparin plasma, EDTA plasma) was dispensed into separate sterile plastic vials (HEME centrifugal tubes, max 1.5 ml). A 0.5 ml aliquot of the standardized *C. albicans* inoculum was aseptically added to each vial, homogenized, and incubated at 37 °C (ROTEX incubator, 230 V, 50 Hz).

Microscopic examination was performed at 60 and 120 minutes. A drop from each vial was placed on a clean HEME glass slide (25.4 × 76.2 mm, 1.0–1.2 mm thick) and covered with a Blue Star cover slip (18 mm). Specimens were observed under a LABOMED microscope at 10× and 45× magnification without staining. Germ tube formation was defined as a filamentous outgrowth from a blastoconidium, ≥ 3 times the length of the parent cell, without constriction at the junction. Germ tube length was measured using an ocular micrometer: a reading of 15–20 µm at 60 minutes and ≥ 40 µm at 120 minutes was considered positive [24].

Statistical Analysis

All data were analysed using appropriate statistical tests. Since germ tube length measurements were obtained from the same 30 samples across three paired groups, the non-parametric Friedman test (equivalent to repeated measures ANOVA) was applied to compare all three media. A paired t-test was used for the two-group comparison between serum and heparin plasma at 120 minutes. A p-value of <0.05 was considered statistically significant.

OBSERVATIONS AND RESULTS

Patient Biochemical Parameters

Biochemical parameters of the 30 study participants were recorded. Blood glucose ranged from 83 to 423 mg/dL, total cholesterol from 137 to 238 mg/dL, and total protein from 6.4 to 9.0 g/dL (Table 1).

Table 1. Patient Biochemical Parameters (n = 30)

S.No	Age/Sex	Glucose (mg/dL)	Cholesterol (mg/dL)	Total Protein (g/dL)
1	71/M	183	206	8.3
2	44/M	90	140	7.9
3	53/M	150	219	8.5
4	48/F	83	163	7.2
5	66/F	158	195	7.2
6	39/M	90	155	8.0
7	64/F	114	209	8.4
8	73/F	122	222	7.6
9	62/M	153	198	7.3
10	70/M	423	163	8.0
11	43/M	143	155	7.9
12	64/M	290	137	6.8
13	44/M	295	159	7.0
14	74/F	104	238	9.0
15	46/M	95	192	8.2
16	71/F	92	168	7.1
17	53/M	105	182	6.8
18	44/M	88	160	7.4
19	47/F	114	195	6.9
20	39/M	99	175	7.2
21	60/M	126	202	6.6
22	77/F	110	190	7.0
23	61/M	98	165	7.3
24	69/F	135	210	6.4
25	48/F	120	185	7.5
26	58/M	140	220	6.7
27	63/M	85	155	7.6
28	65/F	132	205	6.5
29	74/F	108	178	7.0
30	80/M	95	170	6.5

Haematological Parameters — Complete Blood Count

Haematological profiles including differential leucocyte count and platelet count were recorded for all 30 subjects (Table 2).

Table 2. Complete Blood Count — Differential and Platelet Count (n = 30)

S. No	Neutrophil (cells/µL)	Lymphocyte (cells/µL)	Eosinophil (cells/µL)	Monocyte (cells/µL)	Basophil (cells/µL)	Platelet ×10 /µL
1	69	22	4	4	1	1.9
2	57	42	4	4	0	2.6
3	37	58	2	3	0	1.5
4	50	38	8	4	0	2.9
5	57	37	4	2	0	2.3
6	59	36	2	3	0	2.3
7	63	33	1	3	0	2.5
8	62	32	4	2	0	3.9
9	59	23	16	2	0	2.8
10	67	25	3	4	1	4.3
11	76	19	1	4	0	3.9
12	58	34	4	3	0	2.3
13	55	40	2	3	0	2.7
14	51	43	3	3	0	2.4
15	57	35	4	4	0	3.6
16	62	30	3	4	1	2.9
17	58	34	4	3	0	3.1
18	65	28	2	4	0	2.8
19	60	32	3	4	0	3.0
20	55	36	4	4	1	2.7
21	67	25	3	5	1	2.6
22	63	30	3	3	0	2.4
23	57	38	4	5	1	2.9
24	70	22	3	3	0	2.5
25	59	36	5	4	0	3.2
26	72	20	3	3	1	2.3
27	50	44	3	2	1	3.5
28	68	24	2	1	0	2.6

29	61	33	3	3	0	2.4
30	64	30	3	3	1	2.8

Haematological Parameters — Haemoglobin and ESR

Table 3. Haemoglobin and Erythrocyte Sedimentation Rate (n = 30)

S.No	Hemoglobin (g/dL)	ESR (mm/hr)
1	14.3	16
2	15.9	8
3	15.0	12
4	11.7	7
5	13.1	14
6	14.2	12
7	14.1	16
8	12.2	21
9	12.9	7
10	12.0	57
11	11.2	14
12	15.0	5
13	15.3	2
14	13.7	27
15	13.0	18
16	13.4	12
17	12.8	18
18	14.1	10
19	13.0	20
20	12.5	15
21	14.6	22
22	13.7	14
23	12.9	16
24	15.0	24
25	13.2	19
26	14.8	26
27	12.6	13
28	14.3	21
29	13.5	17
30	13.9	12

Germ Tube Formation — Qualitative Results

Germ tube positivity was assessed at 60 and 120 minutes. A reading of 15–20 µm at 60 minutes was considered positive; ≥ 40 µm at 120 minutes was considered positive [24]. All serum and heparin plasma samples were positive in the majority of cases; EDTA plasma was consistently negative (Table 4).

Table 4. Germ Tube Formation-Qualitative Results at 60 and 120 Minutes (n = 30)

S.No	60 Minutes			120 Minutes		
	Serum	Heparin Plasma	EDTA Plasma	Serum	Heparin Plasma	EDTA Plasma
1	Pos	Neg	Neg	Neg	Neg	Neg
2	Pos	Pos	Neg	Pos	Neg	Neg
3	Pos	Pos	Neg	Pos	Pos	Neg
4	Pos	Pos	Neg	Pos	Pos	Neg
5	Pos	Neg	Neg	Neg	Neg	Neg
6	Pos	Pos	Neg	Neg	Pos	Neg
7	Pos	Pos	Neg	Neg	Pos	Neg
8	Pos	Pos	Neg	Pos	Neg	Neg
9	Pos	Pos	Neg	Pos	Pos	Neg
10	Pos	Pos	Pos	Pos	Pos	Neg
11	Pos	Pos	Neg	Pos	Pos	Neg
12	Pos	Pos	Neg	Pos	Pos	Neg
13	Pos	Pos	Neg	Pos	Pos	Neg
14	Pos	Pos	Neg	Pos	Pos	Neg
15	Pos	Pos	Neg	Pos	Pos	Neg
16	Neg	Pos	Neg	Pos	Pos	Neg
17	Neg	Neg	Neg	Neg	Neg	Neg
18	Pos	Neg	Neg	Pos	Pos	Neg
19	Pos	Neg	Neg	Pos	Pos	Neg
20	Pos	Neg	Neg	Neg	Neg	Neg
21	Neg	Neg	Neg	Neg	Neg	Neg
22	Pos	Pos	Neg	Pos	Pos	Neg
23	Neg	Neg	Neg	Neg	Neg	Neg
24	Pos	Neg	Neg	Neg	Pos	Neg
25	Pos	Pos	Neg	Pos	Pos	Neg
26	Pos	Pos	Neg	Pos	Pos	Neg
27	Neg	Neg	Neg	Neg	Neg	Neg
28	Neg	Neg	Neg	Neg	Neg	Neg
29	Pos	Pos	Neg	Pos	Pos	Neg
30	Pos	Pos	Neg	Pos	Neg	Neg

Germ Tube Length - Quantitative Results

Table 5. Germ Tube Length (µm) at 60 and 120 Minutes-All Three Media (n = 30)

S.No	Germ Tube Length at 60 min (µm)			Germ Tube Length at 120 min (µm)		
	Serum	Heparin Plasma	EDTA Plasma	Serum	Heparin Plasma	EDTA Plasma
1	15	10	2	25	25	10
2	15	15	10	40	25	25
3	15	15	2	40	40	25
4	15	15	2	40	40	10
5	15	10	1	25	25	10
6	15	15	2	25	40	15
7	15	15	2	25	40	15
8	15	15	1	40	25	15
9	15	15	2	40	40	15
10	15	15	15	40	40	15
11	15	15	2	40	25	10
12	15	15	2	40	40	15
13	15	15	2	40	40	10
14	15	15	2	40	40	10
15	15	15	2	40	40	15
16	10	15	2	40	40	15
17	10	10	2	25	25	10
18	15	10	2	40	40	15
19	15	10	3	40	40	25
20	15	10	1	25	25	25
21	10	10	1	25	25	15
22	15	15	2	40	40	25
23	10	10	1	25	25	15
24	15	10	2	25	40	15
25	15	15	2	40	40	25
26	15	15	2	40	40	25
27	10	10	1	25	25	10
28	10	10	1	25	25	10
29	15	15	2	40	40	25
30	15	15	2	40	40	25

Statistical Analysis-Friedman Test

Since germ tube length data were obtained from the same 30 samples across three matched groups, the Friedman test (non-parametric equivalent of repeated-measures ANOVA) was applied.

At 60 minutes: Friedman test p-value = 0.000001 ($p < 0.001$). This highly significant result indicates that at 60 minutes, the three media differ substantially: serum produced consistent germ tube lengths (15 µm), heparin plasma produced slightly shorter germ tubes in some samples, and EDTA plasma produced very low germ tube lengths (1–2 µm), indicating strong inhibition of early germ tube formation.

At 120 minutes: Friedman test Chi-square = 26.29; p-value = 0.0000019 ($p < 0.001$). Serum and heparin plasma showed similar germ tube lengths (25–40 µm), while EDTA plasma continued to show significantly lower values (10–15 µm), confirming persistent inhibition.

Serum vs. Heparin Plasma at 120 Minutes -Paired t-Test

Table 6. Germ Tube Length at 120 Minutes - Serum vs. Heparin Plasma (n = 30)

S.No	Serum (µm)	Heparin Plasma (µm)
1	25	25
2	40	25
3	40	40
4	40	40
5	25	25
6	25	40
7	25	40
8	40	25
9	40	40

10	40	40
11	40	40
12	40	40
13	40	40
14	40	40
15	40	40
16	40	40
17	25	24
18	40	40
19	40	40
20	25	25
21	25	25
22	40	40
23	25	25
24	25	40
25	40	40
26	40	40
27	25	25
28	25	25
29	40	40
30	40	40

RESULT:

Paired t-test between serum and heparin plasma germ tube lengths at 120 minutes showed no statistically significant difference ($t = 0.00$; $p = 1.00$). This indicates complete equivalence between the two media, confirming that heparin plasma can reliably replace serum for germ tube testing of *Candida albicans*.

DISCUSSION

The germ tube test remains a cornerstone in the rapid presumptive identification of *Candida albicans* due to its simplicity, cost-effectiveness, and quick turnaround time. This study systematically evaluated heparin plasma and EDTA plasma as alternatives to human serum for germ tube induction, comparing results at two clinically relevant time points (60 and 120 minutes) in 30 patient samples.

Human serum consistently provided optimal conditions for germ tube induction, yielding robust and elongated germ tubes within the diagnostic window. Heparin plasma demonstrated comparable efficiency; germ tubes were observed at both time points and reached lengths statistically equivalent to serum at 120 minutes (paired t-test: $t = 0.00$; $p = 1.00$). This agreement confirms that heparin does not interfere with the physiological processes necessary for germ tube induction — a finding consistent with Moya-Salazar and Rojas [6] and Hidayati et al. [11]. Crucially, heparin plasma offers practical advantages: it eliminates the need for clot formation and additional centrifugation steps, reduces biohazard exposure from fresh serum handling, and can be derived from routine haematology tubes already available in the laboratory workflow [12].

In contrast, EDTA plasma exhibited a pronounced inhibitory effect on germ tube formation. This is attributable to EDTA's well-established chelating action on divalent cations (Ca^{2+}, Mg^{2+}), which are essential cofactors for the enzymatic and signalling pathways governing *C. albicans* morphogenesis and the yeast-to-hypha transition [11]. Even at 120 minutes, EDTA plasma failed to support true germ tube formation in most samples, with lengths remaining well below the positive threshold. The use of EDTA plasma risks false-negative results and potentially delays diagnosis and the initiation of antifungal therapy-outcomes of particular concern in immunocompromised patients.

Biochemical parameters (blood glucose, cholesterol, total protein) and haematological values (CBC, Hb, ESR) showed no significant correlation with germ tube formation in any medium tested. Notably, one patient with a markedly elevated blood glucose of 423 mg/dL (consistent with diabetic candidaemia) did not exhibit any aberrant germ tube response, indicating that the germ tube test's reliability with serum and heparin plasma is robust against common metabolic variations [22, 23].

The Friedman test confirmed highly significant overall differences among the three media at both time points (60 min: $p < 0.001$; 120 min: $p < 0.001$), driven primarily by EDTA plasma's persistent suppression of germ tube development. These results align with UK SMI guidelines, which emphasise completing germ tube assessments within 2–3 hours to minimise false positives from non-albicans species — a window reliably met by serum and heparin plasma, but not by EDTA plasma [12]. Taken together, these findings strongly support adopting heparinized plasma as a standard alternative in routine microbiology laboratories, particularly in resource-limited or biosafety-sensitive settings.

CONCLUSION

This study demonstrates that heparin plasma is a statistically equivalent, safer, and more practical alternative to human serum for germ tube induction in *Candida albicans*.

Serum and heparin plasma support germ tube formation equally well, with no statistically significant difference in germ tube length at 120 minutes ($t = 0.00$; $p = 1.00$).

EDTA plasma significantly suppresses germ tube formation due to its chelating effect on essential divalent cations (Ca^{2+}, Mg^{2+}) and must not be used for this purpose.

Heparin plasma can be recommended as a reliable, cost-effective, and biosafe substitute for serum in routine clinical microbiology laboratories.

Patient biochemical and haematological parameters do not significantly influence in vitro germ tube induction in serum or heparin plasma.

Implementation of heparin plasma-based germ tube testing can streamline laboratory workflow, reduce biosafety risks, and facilitate faster presumptive identification of Candida albicans, contributing to earlier antifungal intervention and improved patient outcomes.

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