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Hydatidiform mole presenting with a negative urine pregnancy test and extremely high serum β-hCG: A case report of the high-dose hook effect

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Keywords

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Abstract

Hydatidiform mole is a form of gestational trophoblastic disease characterized by abnormal proliferation of trophoblastic tissue and excessive production of human chorionic gonadotropin (hCG). Although markedly elevated β-hCG concentrations are characteristic of molar pregnancy, extremely high concentrations may paradoxically result in falsely low or negative results in some immunometric assays. This analytical phenomenon, known as the high-dose hook effect or prozone phenomenon, can create a significant diagnostic dilemma.A 20-year-old woman presented to the emergency department with acute, non-radiating lower abdominal pain. Her vital signs were stable, and a lower abdominal mass was palpable. Menstrual history was reported as regular, although the patient was unable to recall her last menstrual period. Per-vaginal examination revealed a uterus corresponding to approximately 26 weeks of gestation. Repeated qualitative urine β-hCG tests were negative. Ultrasonography subsequently demonstrated an enlarged uterus with a disorganized/distorted endometrial cavity suggestive of a complete hydatidiform mole. Laboratory investigations revealed severe anemia with a hemoglobin concentration of 5.5 g/dL. In view of the clinical and ultrasonographic findings, quantitative serum β-hCG was performed and was extraordinarily elevated at 12,627,000 mIU/mL.The combination of clinical findings, ultrasonographic features, and markedly elevated serum β-hCG established the diagnosis of hydatidiform mole despite repeatedly negative urine pregnancy tests. The patient received blood transfusion for severe anemia and subsequently underwent suction evacuation. Serum β-hCG decreased to 744,040 mIU/mL following evacuation, and follow-up was planned for detection of persistent or recurrent disease.A negative urine pregnancy test does not exclude pregnancy or gestational trophoblastic disease when clinical suspicion is high. The high-dose hook effect should be considered when a markedly elevated hCG-producing condition is suspected but an immunoassay result is unexpectedly negative or disproportionately low. In such situations, quantitative serum β-hCG testing, appropriate sample dilution, and communication between the clinical and laboratory teams are essential to prevent diagnostic delay.

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1. Introduction

Hydatidiform mole is a premalignant form of gestational trophoblastic disease characterized by abnormal proliferation of trophoblastic tissue and hydropic degeneration of chorionic villi. It is classified into complete and partial hydatidiform mole. Complete moles are typically associated with substantially increased hCG concentrations because of extensive trophoblastic proliferation (Ngan et al. 2021).

The clinical presentation of molar pregnancy has changed with the increasing use of early ultrasonography. Classical features such as vaginal bleeding, excessive uterine enlargement, hyperemesis, preeclampsia, hyperthyroidism, and passage of grape-like vesicles are now less frequently encountered because many cases are diagnosed during the first trimester. Nevertheless, markedly elevated hCG concentrations remain an important laboratory feature of complete molar pregnancy (Ngan et al. 2021; Patil BU, 2020).

Human chorionic gonadotropin is commonly detected using immunometric sandwich assays. These assays generally employ two antibodies directed against different epitopes of the analyte. At appropriate concentrations, the antigen forms a sandwich between the capture and detection antibodies, generating a measurable signal. However, when the concentration of the analyte becomes extremely high, antibody binding sites may become saturated independently, interfering with formation of the antigen–antibody sandwich. The resulting signal may paradoxically decrease, producing a falsely low or even negative result. This phenomenon is known as the high-dose hook effect or prozone phenomenon (Mina et al. 2024).

The hook effect is particularly important in conditions associated with extremely high concentrations of hCG, including advanced hydatidiform mole and gestational trophoblastic neoplasia. Recent reports have demonstrated that dilution of the specimen can overcome this analytical interference and reveal the true hCG concentration (Phillipo et al. 2024; Yang et al. 2025).

We report a case of hydatidiform mole in a young woman who repeatedly had negative qualitative urine pregnancy tests despite an extremely high serum β-hCG concentration. The case highlights the importance of recognizing analytical interference when laboratory results are discordant with the clinical and imaging findings.

Case Presentation

A 20-year-old woman presented to the emergency department with acute lower abdominal pain. The pain was non-radiating and was not relieved by medication. On examination, she was hemodynamically stable, with a pulse rate of 75 beats/minute and blood pressure of 110/80 mmHg. A lower abdominal mass was palpable.

The patient was unable to recall her last menstrual period. She reported a menstrual pattern described in the original clinical record as regular. Per-vaginal examination revealed an enlarged uterus corresponding to approximately 26 weeks of gestation.

Pregnancy was initially considered, and a qualitative urine β-hCG test was performed. Surprisingly, the test was negative. The urine pregnancy test was repeated on more than one occasion to exclude possible technical errors, including mislabeling or incorrect test performance, but the results remained negative.

Because of the enlarged uterus and palpable abdominal mass, ultrasonography was performed. The scan demonstrated an enlarged uterus with a distorted/disorganized endometrial cavity, with features suggestive of a complete hydatidiform mole (Figure 1).



Figure 1: Gross appearance of H mole (grape like) on expulsion

The discordance between the clinical and ultrasonographic findings and the negative urine pregnancy tests prompted further laboratory investigation.

Laboratory Investigations

Routine investigations were performed. The most significant abnormality was severe anemia, with a hemoglobin concentration of 5.5 g/dL.

Quantitative serum β-hCG was subsequently requested

and showed an extraordinarily high concentration:

Serum β-hCG: 12,627,000 mIU/mL

The assay was performed using the VITROS ECiQ system using Vitros β-hCG II test based on immunometric immunoassay method using serum as sample with a reportable range of 2.39- 15,000mIU/Ml.

Thyroid function tests showed:

- Free T3: 23 pmol/L
- Free T4: 77.1 pmol/L
- TSH: 0.015 mIU/L suggesting thyrotoxicosis.

The laboratory findings were compatible with an hCG-secreting gestational trophoblastic disorder. The markedly elevated serum β-hCG, together with the ultrasonographic appearance of the uterus, supported the diagnosis of hydatidiform mole.

Table 1. Summary of clinical and laboratory findings

Parameter	**Finding**
Age	20 years
Presenting symptom	Acute lower abdominal pain
Pulse	75 beats/minute
Blood pressure	110/80 mmHg
Uterine size	Approximately 26 weeks
Urine qualitative β-hCG	Negative repeatedly
Ultrasonography	Enlarged uterus with distorted/disorganized endometrial cavity suggestive of complete hydatidiform mole
Hemoglobin	5.5 g/dL
Serum β-hCG	12,627,000 mIU/mL
Free T3	23 pmol/L
Free T4	77.1 pmol/L
TSH	0.015 mIU/L
Management	Blood transfusion followed by suction evacuation
Post-evacuation β-hCG	744,040 mIU/mL

The original case record documents the above investigations and the subsequent reduction in serum β-hCG following evacuation.

Differential Diagnosis

The initial differential diagnosis of a markedly enlarged uterus and lower abdominal mass included:

- Hydatidiform mole
- Pregnancy
- Degenerating uterine fibroid
- Ectopic pregnancy
- Multiple gestation
- Other uterine masses
- Pregnancy-associated or trophoblastic malignancy

The repeatedly negative urine pregnancy test initially reduced clinical suspicion for pregnancy-related pathology. However, the ultrasonographic findings

prompted quantitative serum β-hCG testing, which revealed the extreme elevation characteristic of an hCG-producing trophoblastic disorder.

Management and Outcome

In view of the severe anemia, the patient was initially managed with blood transfusion. Following appropriate clinical assessment and preparation, she underwent suction evacuation for hydatidiform mole (Picture 1).

Following evacuation, the serum β-hCG concentration decreased from 12,627,000 mIU/mL to 744,040 mIU/mL.

The patient was subsequently planned for follow-up to monitor the endometrium and assess for persistence or recurrence of gestational trophoblastic disease. Serial hCG monitoring is an essential component of follow-up after molar pregnancy because persistent or rising hCG may indicate post-molar gestational trophoblastic neoplasia (Ngan et al. 2021).

Discussion

The most important feature of this case is the striking discrepancy between the clinical findings, ultrasonographic findings, and the initial urine pregnancy test. The patient had a uterus corresponding to approximately 26 weeks of gestation and ultrasound findings suggestive of a complete hydatidiform mole, yet repeated qualitative urine β-hCG tests were negative. Subsequent quantitative serum testing revealed an extraordinarily high β-hCG concentration of 12,627,000 mIU/mL.

This apparent contradiction is explained by the high-dose hook effect.

The high-dose hook effect

Most qualitative pregnancy tests and many quantitative hCG assays are based on immunometric sandwich principles. The analyte is captured by one antibody and detected by a second labeled antibody (Figure 1). Under normal circumstances, increasing analyte concentration results in increasing formation of antibody–antigen–antibody complexes and therefore an increasing signal.

At extremely high analyte concentrations, however, this relationship can become nonlinear. Excess antigen may saturate the capture and detection antibodies separately, preventing adequate formation of the sandwich complex. Consequently, the measured signal falls despite the actual concentration of analyte being extremely high. The result can therefore be falsely low or negative (Figure 2).

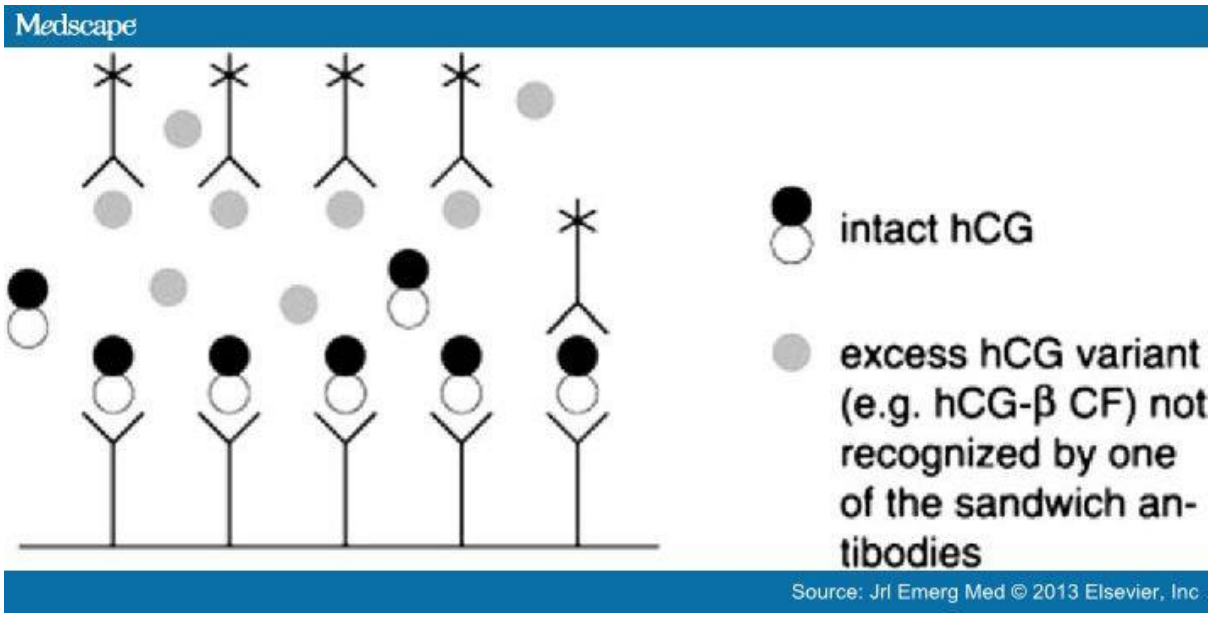


Figure 2: Showing capturing of antibodies

This phenomenon is referred to as the high-dose hook effect or prozone phenomenon. It has been reported with several immunoassays, including assays for hCG, prolactin, prostate-specific antigen, ferritin, CA-125, and other analytes (Mina et al. 2024).

The laboratory should therefore recognize that an unexpectedly low or negative result does not necessarily indicate absence of the analyte. When the clinical suspicion is strong and the laboratory result is inconsistent with the clinical picture, analytical interference should be considered.

Hook effect in hydatidiform mole

Hydatidiform mole is particularly relevant because trophoblastic tissue can produce very large quantities of hCG. The concentration may reach several million IU/L in advanced disease (Figure 3). At such concentrations, some hCG immunoassays can demonstrate a high-dose hook effect (AACC Academy, 2022).

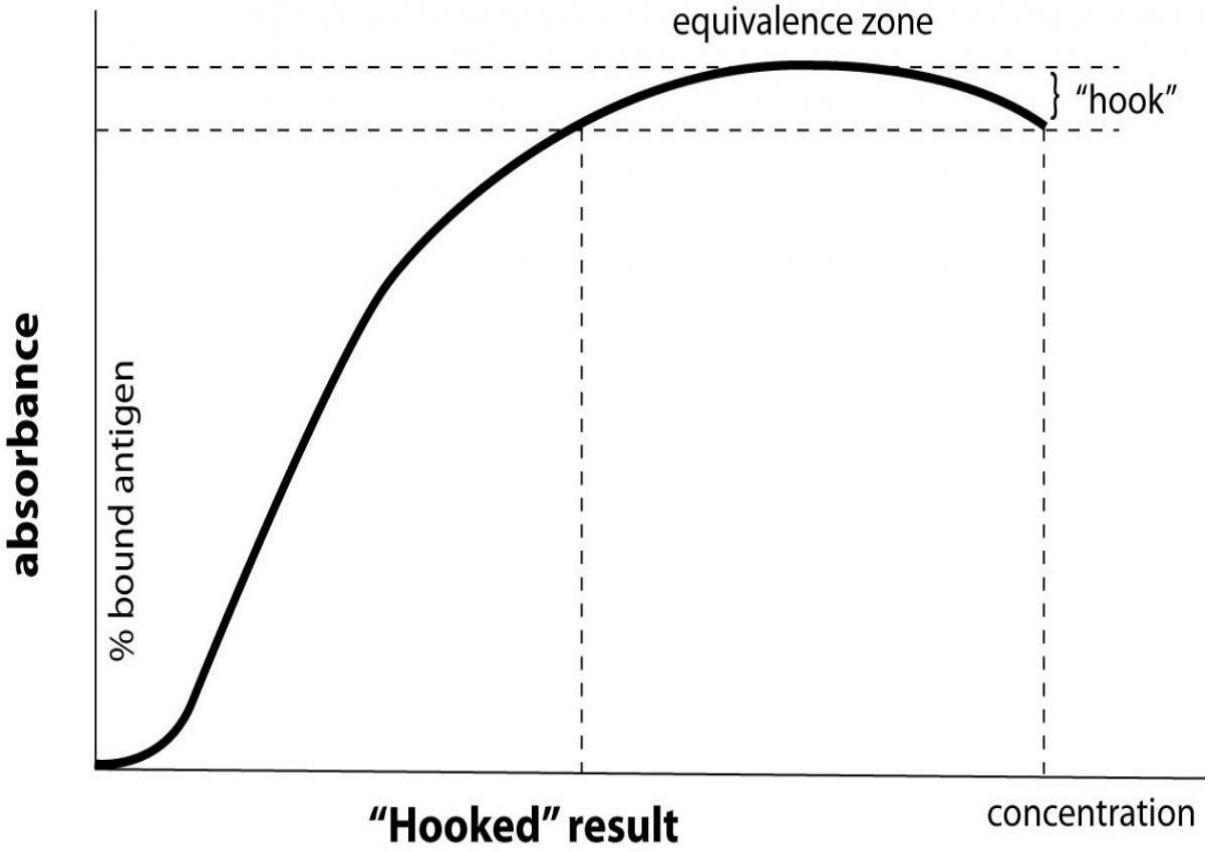


Figure 3: Describes about the Hook effect

Several case reports have described false-negative or falsely low hCG results in patients with molar pregnancy. Phillipo et al. (2024), for example, reported a patient with classical ultrasonographic findings of complete molar pregnancy and a false-negative qualitative urine hCG result. The report emphasized the importance of considering the hook effect when clinical findings and hCG results are discordant.

Similarly, a 2024 report described hook effect-associated falsely low hCG results in a patient with gestational trophoblastic disease. The authors emphasized the importance of laboratory investigation when the hCG concentration does not fit the clinical presentation (Heda et al. 2024).

A recent 2025 report further demonstrated that serial dilution can reveal the true β-hCG concentration in patients with hydatidiform mole and suspected hook effect (Yang et al. 2025).

Importance of sample dilution

Sample dilution is one of the simplest and most effective approaches for investigating a suspected hook effect. Dilution reduces the analyte concentration into the analytical measurement range and allows the antibodies to interact appropriately with the antigen. If a hook effect is present, the measured concentration after dilution may be disproportionately higher than the undiluted result.

Recent laboratory literature recommends considering dilution when there is clinical suspicion of an extremely high analyte concentration or when an immunoassay result is inconsistent with the clinical findings (Mina et

al. 2024, Gupta et al 2021).

An important practical point is that the susceptibility to the hook effect is **assay dependent**. Different analyzers and manufacturers may demonstrate different thresholds for the phenomenon. Consequently, laboratory personnel should be familiar with the manufacturer's stated analytical limitations and, when necessary, investigate a discrepant result using dilution or an alternative analytical platform (CLSI-2012).

Clinical significance of a negative pregnancy test

A negative urine pregnancy test is generally reassuring in routine clinical practice, but it should not be regarded as definitive evidence against pregnancy when the pretest probability is high.

In this case, the negative urine test could have resulted in diagnostic anchoring and a delay in considering pregnancy-related pathology. The subsequent ultrasound demonstrated findings suggestive of hydatidiform mole, prompting quantitative serum β-hCG measurement.

This illustrates an important principle of laboratory medicine:

Laboratory results must always be interpreted in the context of the clinical picture.

When laboratory results and clinical findings are markedly discordant, repeating the test alone may not be sufficient. The laboratory should consider analytical interference, specimen dilution, an alternative assay platform, and discussion with the treating clinician.

Hydatidiform mole and hCG-associated complications

Markedly elevated hCG concentrations in complete hydatidiform mole may also have systemic effects. hCG has structural similarity to thyroid-stimulating hormone and, at very high concentrations, can stimulate the TSH receptor. Consequently, biochemical or clinical hyperthyroidism may occur in patients with extensive trophoblastic disease.

In the present case, the thyroid profile showed elevated free T3 and free T4 with suppressed TSH, providing biochemical evidence of thyroid stimulation in the setting of extreme hCG elevation.

Current approaches to gestational trophoblastic disease emphasize early diagnosis, appropriate evacuation, and serial hCG monitoring after treatment (Ngan et al. 2021). Persistent or rising hCG after evacuation requires evaluation for post-molar gestational trophoblastic neoplasia.

Laboratory Medicine Perspective

This case provides several important lessons for clinical laboratories.

First, **a negative qualitative test should not automatically be interpreted as absence of the analyte** when the clinical probability is high.

Second, an unexpectedly negative or low result in a patient with an apparent hCG-producing disorder should prompt consideration of the **high-dose hook effect**.

Third, the laboratory should consider performing **serial dilution of the specimen**. A rising result after appropriate dilution strongly supports the presence of an assay-related hook effect.

Fourth, the use of an **alternative assay platform** can sometimes help identify method-specific susceptibility to interference.

Finally, close communication between the clinician and laboratory is essential. Modern immunoassays are highly automated and generally reliable, but analytical limitations remain clinically important. Recent literature emphasizes that hook effect can result in potentially consequential diagnostic errors if not recognized (Mina et al. 2024; Yang et al. 2025).

Strengths and Limitations of the Case

The major strength of this case is the extreme discordance between the repeated negative urine pregnancy tests and the extraordinarily high quantitative serum β-hCG concentration. The serum β-hCG concentration of 12,627,000 mIU/mL provides a striking illustration of the potential for analytical interference in hCG immunoassays.

A limitation is that the original clinical record does not provide detailed documentation of the serial dilution results for the urine or serum sample. Therefore, although the clinical presentation is highly consistent with a high-dose hook effect, the reconstructed report should avoid stating that a dilution study definitively demonstrated the phenomenon unless the original laboratory records confirm this.

Similarly, histopathological findings are not documented in the supplied case presentation. Therefore, the diagnosis is described as being supported by the clinical, biochemical, and ultrasonographic findings rather than claiming a histopathologically confirmed complete mole.

Conclusion

Hydatidiform mole may present with an apparently paradoxical laboratory finding: a negative urine pregnancy test despite extraordinarily high serum β-hCG concentrations.

The high-dose hook effect is an important analytical phenomenon that can produce falsely low or negative results in sandwich immunoassays when analyte concentrations are extremely high. In patients with clinical or ultrasonographic findings suggestive of molar pregnancy, a negative urine pregnancy test should therefore not be considered sufficient to exclude pregnancy or gestational trophoblastic disease.

When there is a discrepancy between clinical findings and laboratory results, quantitative serum β-hCG measurement, appropriate specimen dilution, review of assay characteristics, and, where necessary, testing on an alternative analytical platform should be considered.

The central message from this case is that **an unexpected laboratory result should trigger investigation of the result itself, rather than immediate dismissal of the clinical diagnosis**.

Key Learning Points

1. A negative urine pregnancy test does not completely exclude pregnancy or gestational trophoblastic disease.
2. Extremely high hCG concentrations can produce falsely low or negative results because of the high-dose hook effect.
3. The hook effect is an important limitation of two-site immunometric sandwich assays.
4. A clinical–laboratory discrepancy should prompt communication between the treating clinician and laboratory.
5. Serial dilution of the specimen can help identify and overcome the hook effect.

6. Assay susceptibility to the hook effect may vary between analytical platforms.
7. Quantitative serum β-hCG and ultrasonography are important when molar pregnancy is suspected despite a negative qualitative pregnancy test.
8. Serial hCG monitoring after evacuation is essential for detection of persistent gestational trophoblastic disease.

Declarations

Patient Consent

Written informed consent was obtained from the patient for publication of the case.

Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

Laboratory investigation, interpretation of laboratory findings, diagnosis, literature review, and manuscript preparation (KB), Clinical management and obstetric and gynecological evaluation (AT), manuscript review and editing (PU).

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