State-of-the-Art Blocking of False Positives

Our mission is to
Inform about the **origin**
Alert about the **prevalence**
Warn about the **consequences**
... of false positive immunoassay test results and ultimately assist people in reducing this very serious problem. Scantibodies Laboratory, Inc. is committed to improving patient outcomes by contributing to the process of accurately diagnosing diseases to lead to cures.

- What is a false positive?
- Where do heterophilic false positives come from?
- What is the prevalence of the heterophilic false positive?
- What are the consequences of false positives?
- How can a false positive be identified?
- How does the heterophilic antibody cause a false positive test result?
- How can the heterophilic false positive be prevented?
- What is the typical cost to block a heterophilic false positive?
- What can the assay manufacturer do?
- What can the clinical lab do?
- How to reduce customer complaints with HBT?

The Definition

False lab test results may be referred to as . . .

False Positive

A lab result indicating a certain analyte is present, when, in fact ***IT IS NOT.***

or

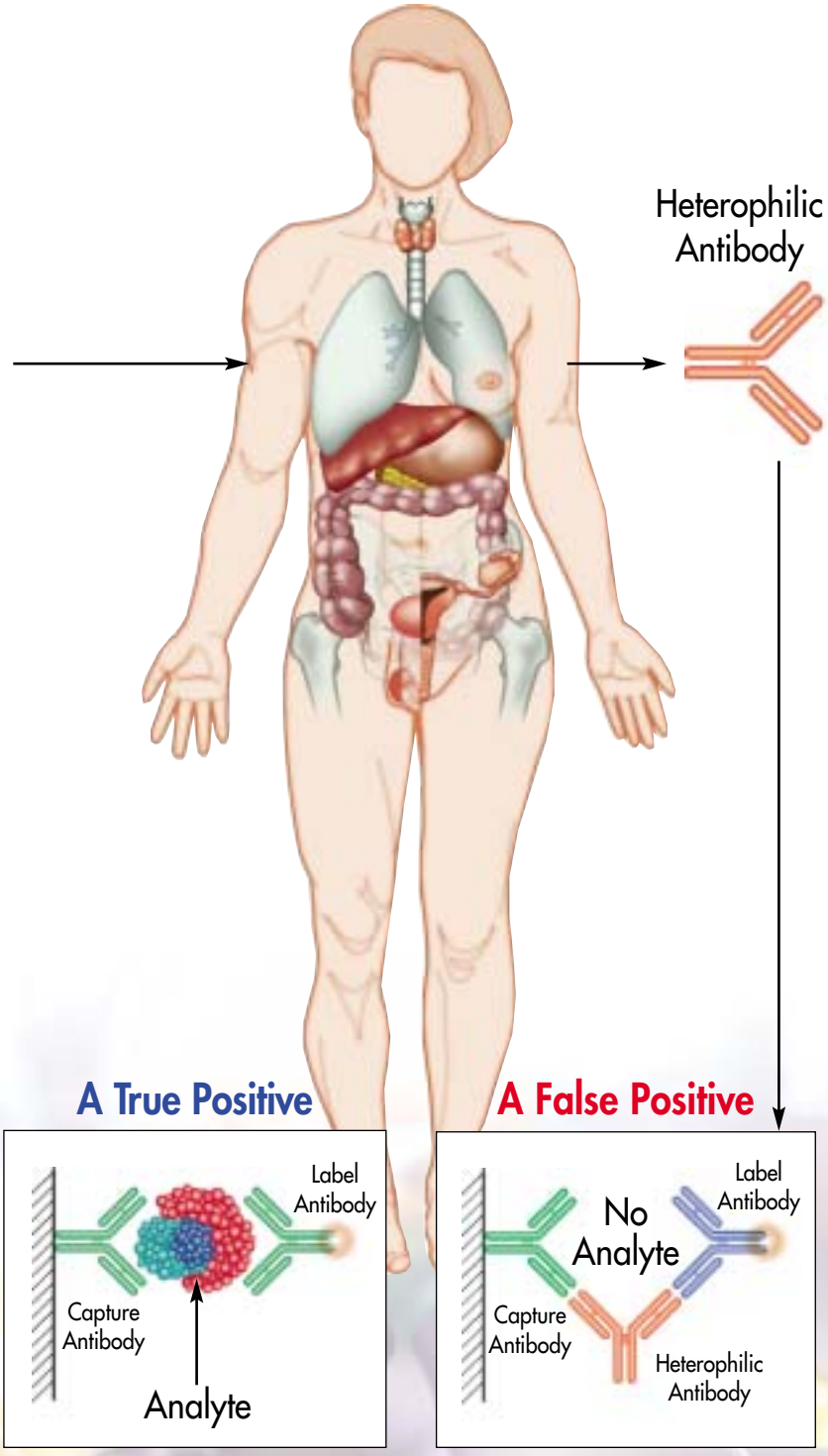
False Negative

A lab result indicating a certain analyte is not present, when in fact ***IT IS.***

The Heterophilic Antibody

Where it comes from . . .
How it can cause a False Positive

- Rheumatoid Arthritis
- Vaccinations
- Influenza
- Animal Contact (Pets)
- Allergies
- Special Diets (e.g., Cheese,)
- Blood Transfusions
- Alternate Animal Contact Therapy (e.g., Thymic Cells, Sheep Cells, Embryonic Cells)
- Autoimmune Diseases
- Dialysis
- Patent Medicines (OKT3)
- Maternal Transfer
- Cardiac Myopathy
- G.I. Disease (E. Coli)



The heterophilic antibody causes a False Positive Test Result by cross bridging the Capture and Label Assay Antibodies

Animal-derived pharmaceuticals

Drug	Source	Ref.#
Antibody-targeted imaging reagents	Mouse	23
	Rat	24
Antibody-targeted drugs	Mouse	23
	Rat	24
Anti-thymocyte globulin	Horse	25
	Rabbit	26
Anti-snake venom	Horse	27
Calcitonin	Salmon	28
Digibind (anti-digoxin Fab)	Sheep	29
Factor VIII	Pig	30
Insulin	Pig	31
Vaccines	Rabbit	32
	Chicken	33
Patent Medicines	Rabbit	34

Ref: Kricka, Larry J., "Human Anti-Animal Antibody Interferences in Immunological Assays," *Clin Chem 45:7, 942-956 (1999)*

What is the prevalence of the heterophilic false positive antibody in patients?

"Endogenous human heterophilic antibodies which have the ability to bind to immunoglobulins of other species are present in the serum or plasma of more than 10% of patients."

– College of American Pathologists

"10% - 40% of the population may experience HAMA interference (depending on the design of the assay used to detect HAMA interference)."

– Larry Kricka, President, AACC

"A study involving 500 patients looked at thyroid-stimulating hormone and gonadotropins, and there the percentage of incorrect results was 0.5 percent."

– Larry Kricka, President, AACC

Scantibodies False Positive Research

With an ultimate goal of gaining a better understanding of:

- The extent of the false positive problem
- The nature of the false positive interference
- The best means of preventing false positive interferences

Scantibodies performs false positive research services such as:

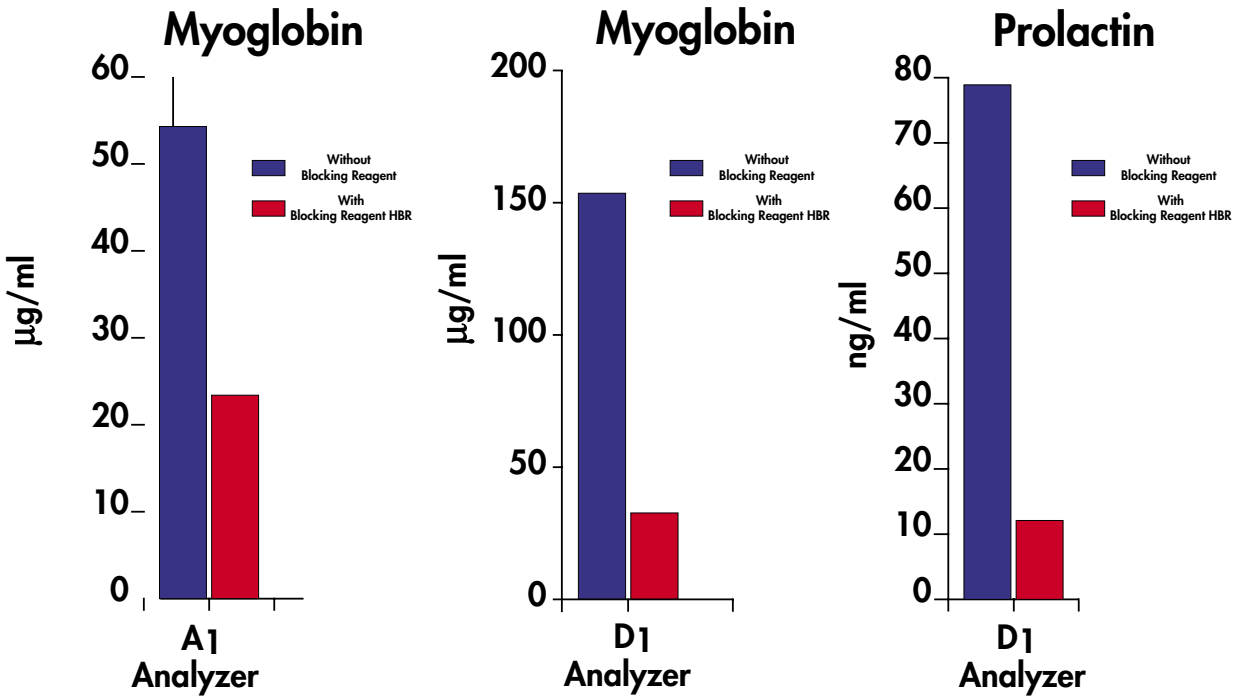
IMMUNOASSAY FALSE POSITIVE BASIC EXAMINATION RESEARCH SERVICE

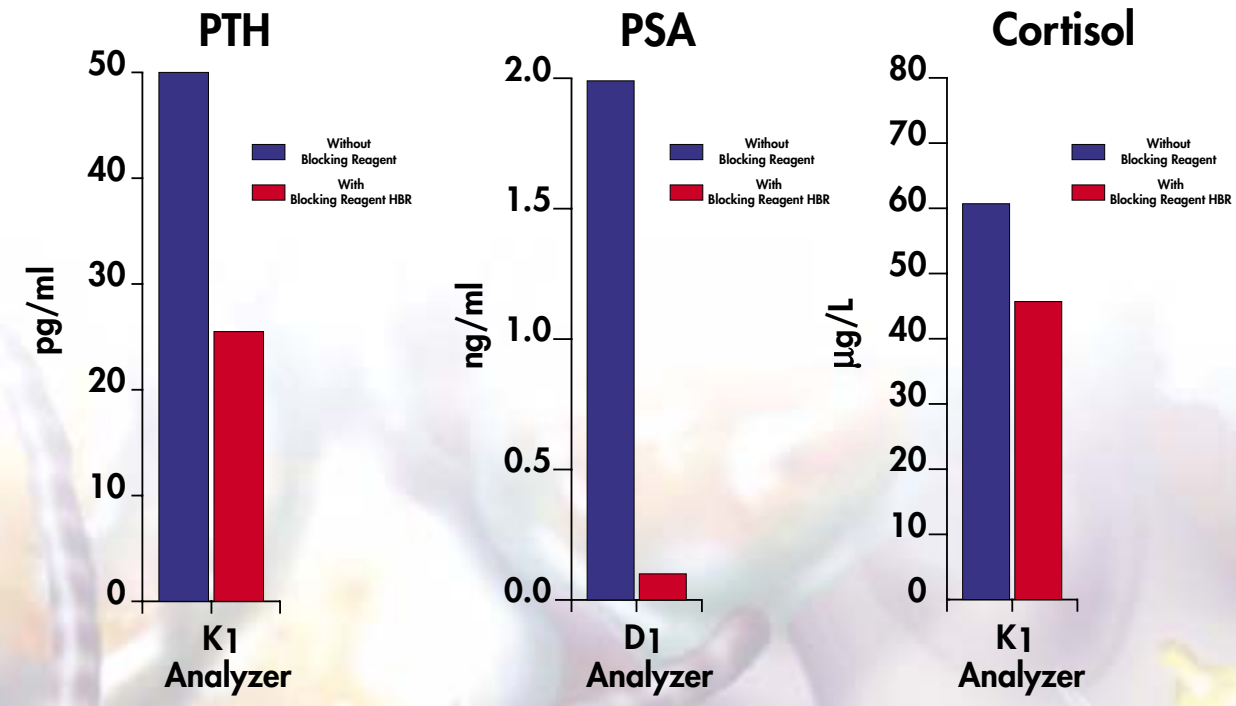
SCANTIBODIES → Sends a false positive heterophilic sample → Lab → The sample is assayed in all of the lab's routine immunoassays → If an assay generates a positive result → Confirmation

- 10,022 Clinical labs contacted.
- 1850 Books sent out.
- 84 False positive sample panels sent.
- 65 Assay systems results reported.
- 56 Systems tested and 41% had at least one false positive or false elevation.
- 334 Assay tests and 16.5% generated a false positive or false elevation.
- 76 Analytes tested generated 44.7% false positive or false elevation.

False Positive Studies in Immunoassay Systems using one Heterophilic Antibody Positive Sample

Donor #1





Note: Plateau Values were not established for the above blockers. If more HBR were used, values would decrease further.

False Positive Studies in Immunoassay Systems using one Heterophilic Antibody Positive Sample

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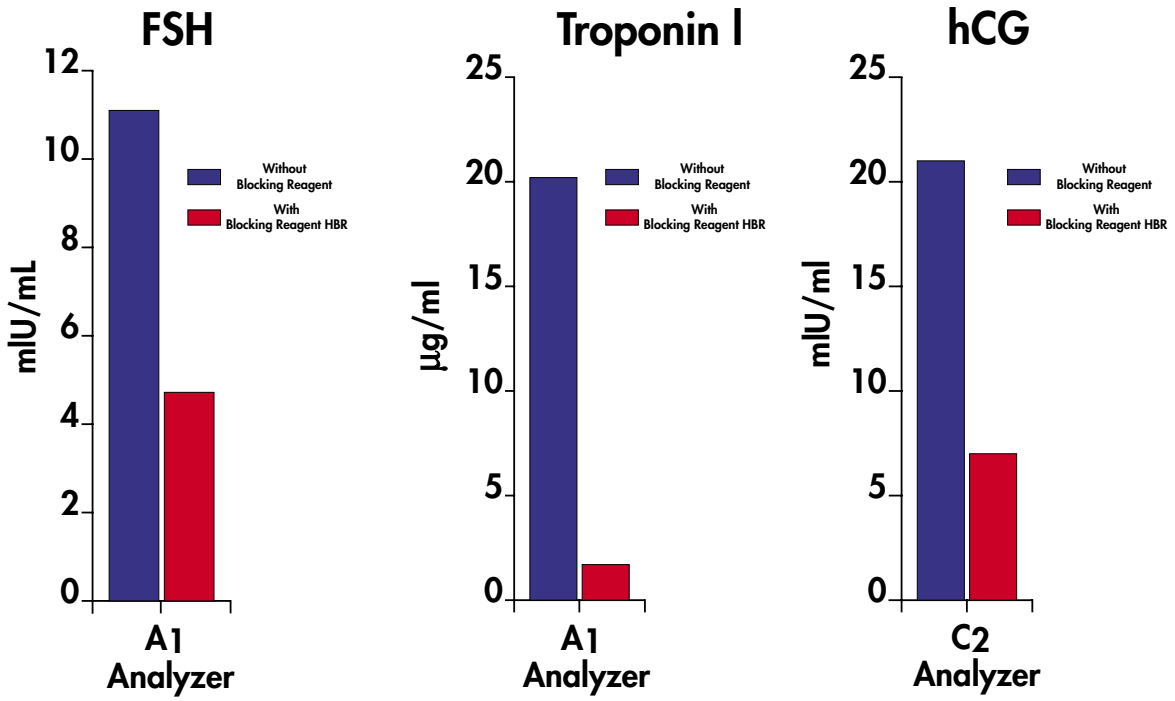

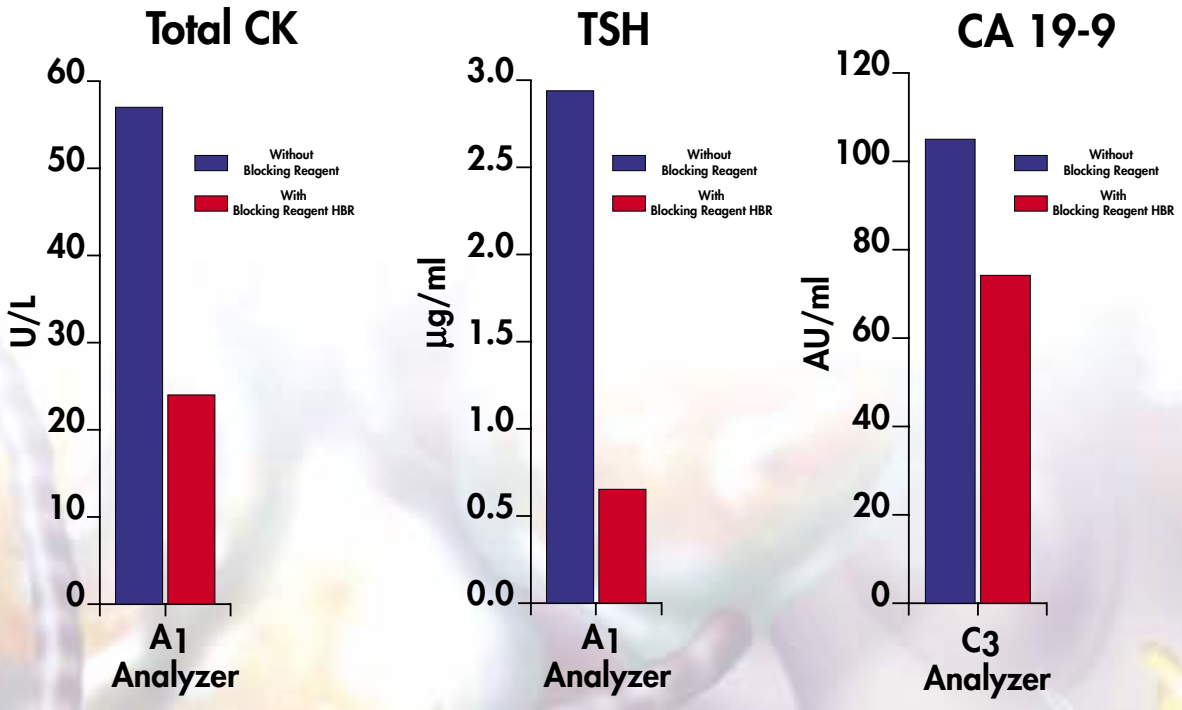

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False Positive Studies in Immunoassay Systems using one Heterophilic Antibody Positive Sample

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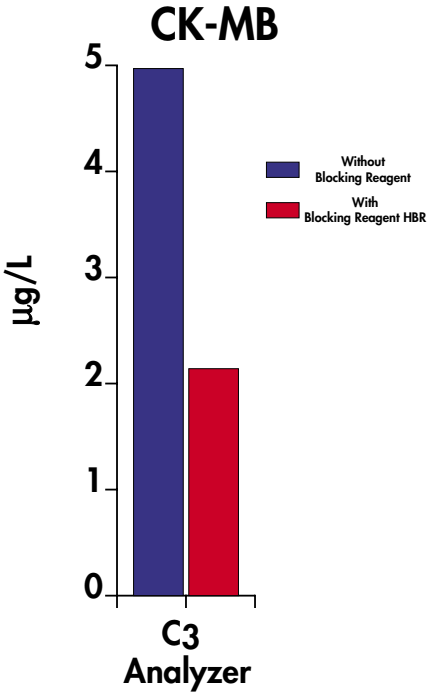

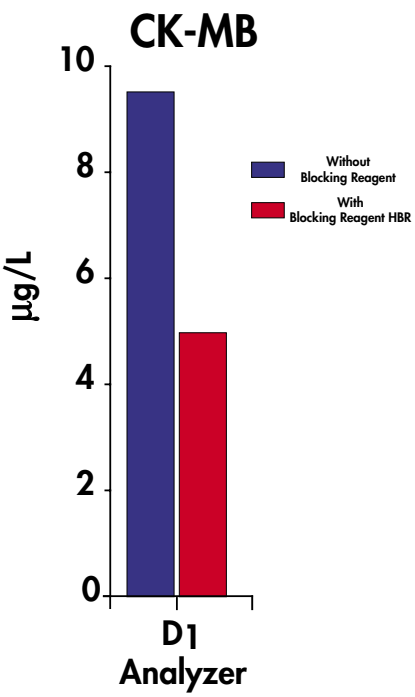

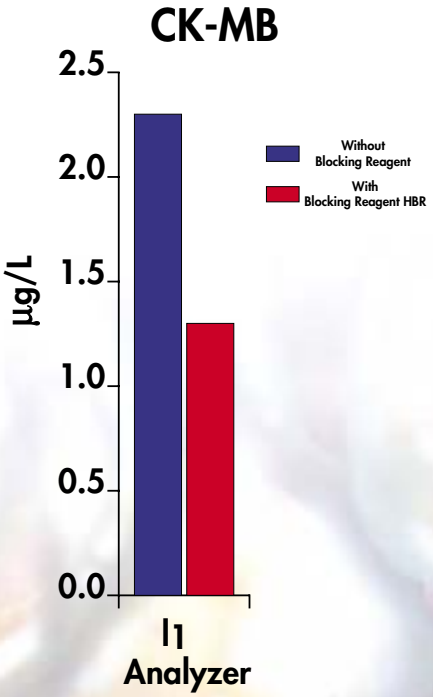

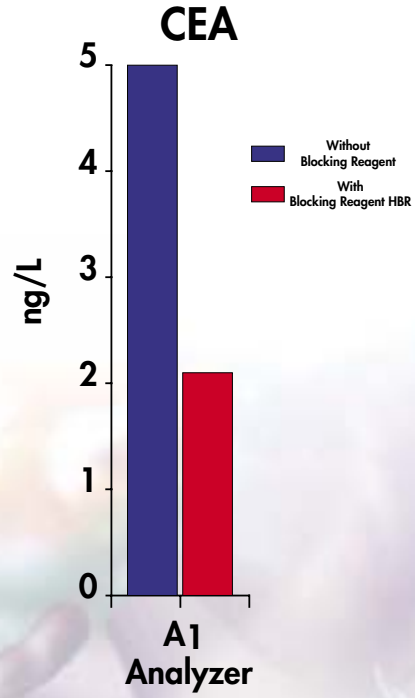

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False Positive Studies in Immunoassay Systems using one Heterophilic Antibody Positive Sample

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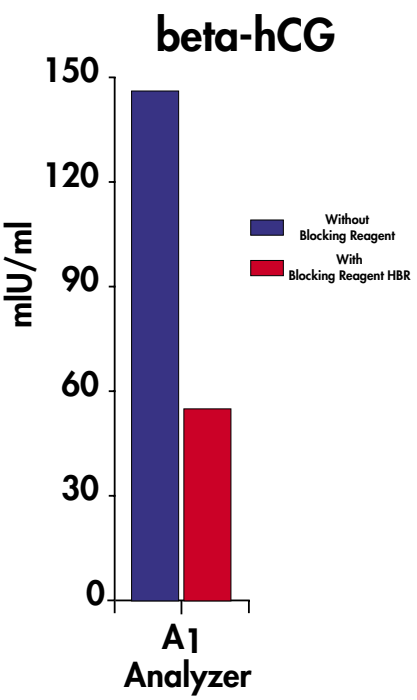

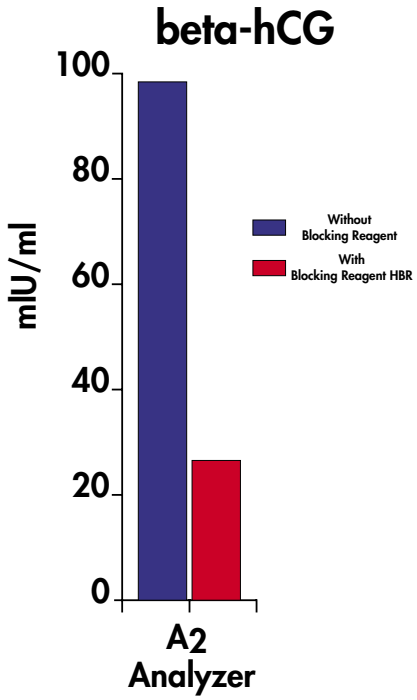

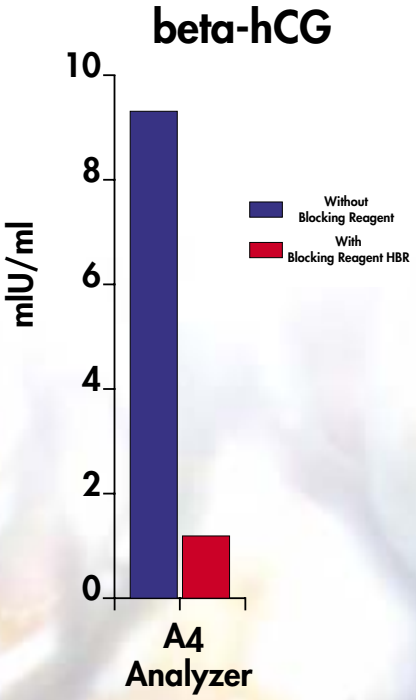

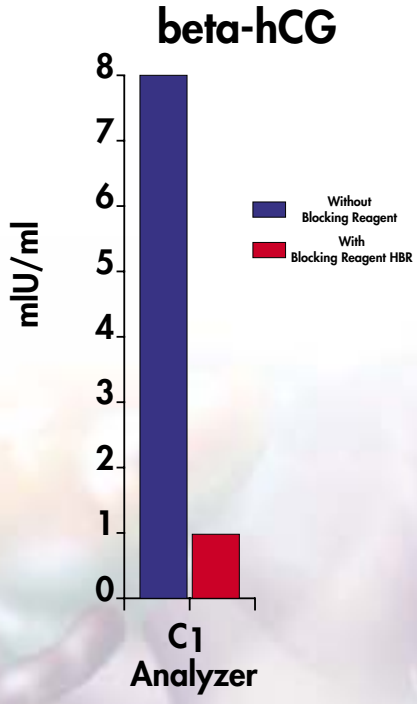

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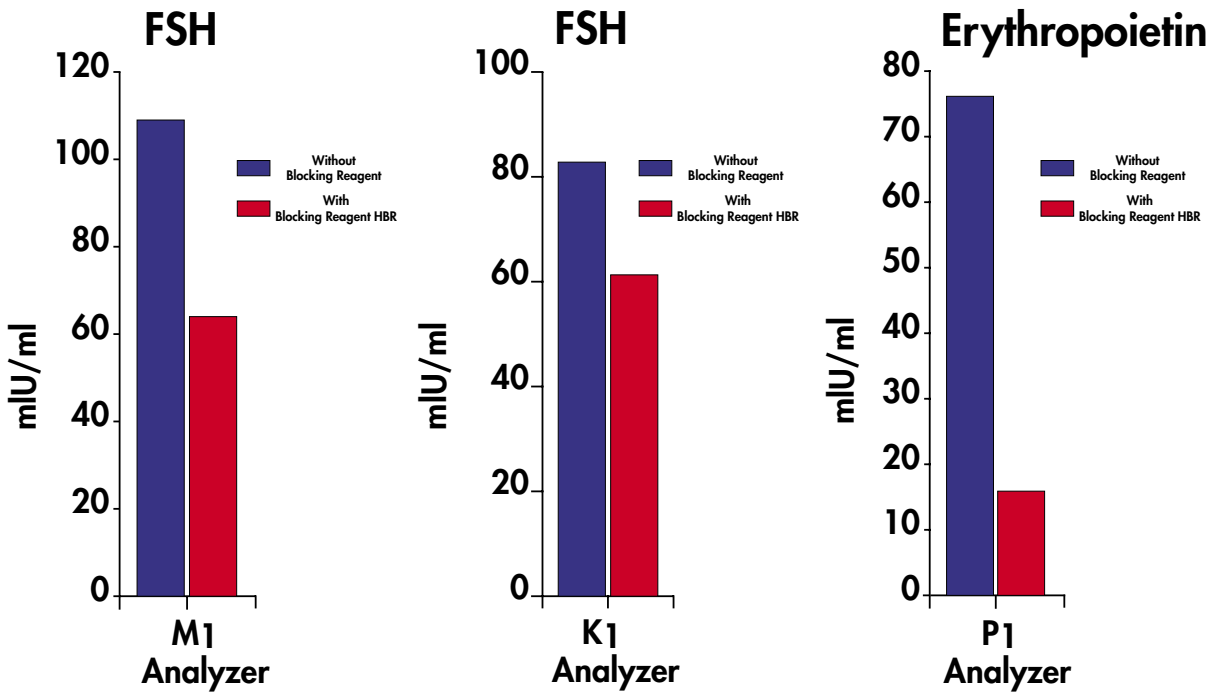
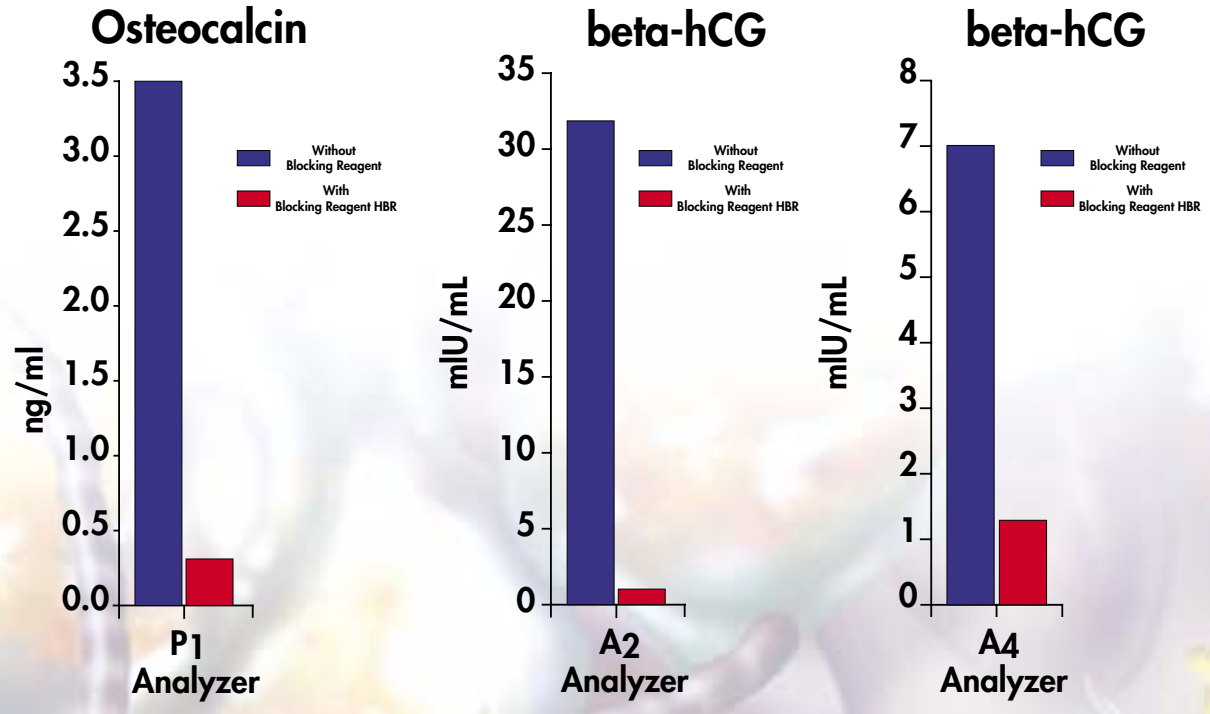
continued

False Positive Studies in Immunoassay Systems using one Heterophilic Antibody Positive Sample

Donor #2





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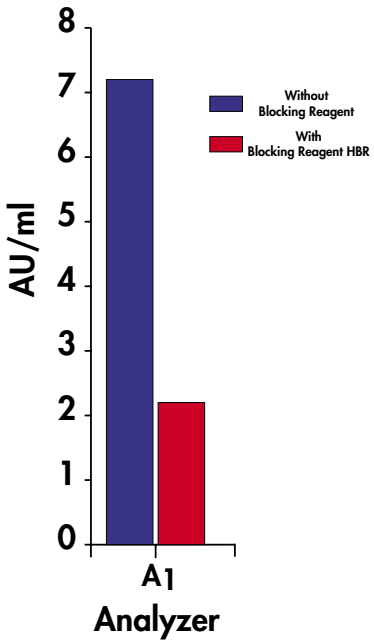
continued

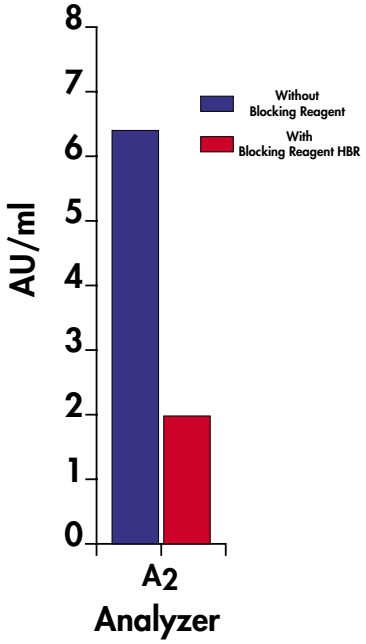
False Positive Studies in Immunoassay Systems using one Heterophilic Antibody Positive Sample

Donor #2

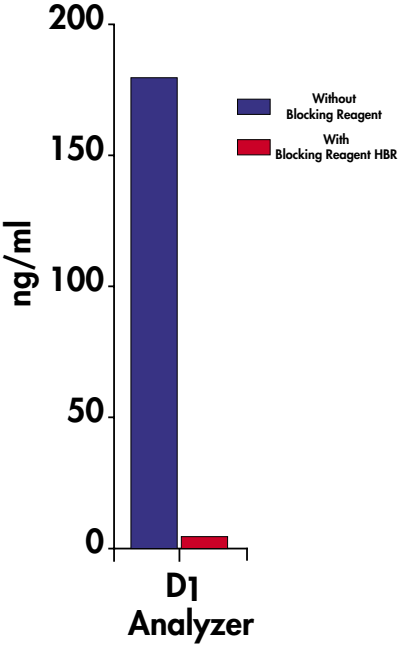
CA 19-9



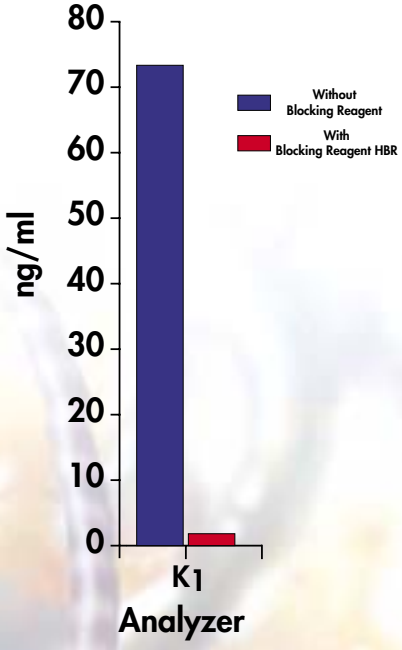
CA 19-9



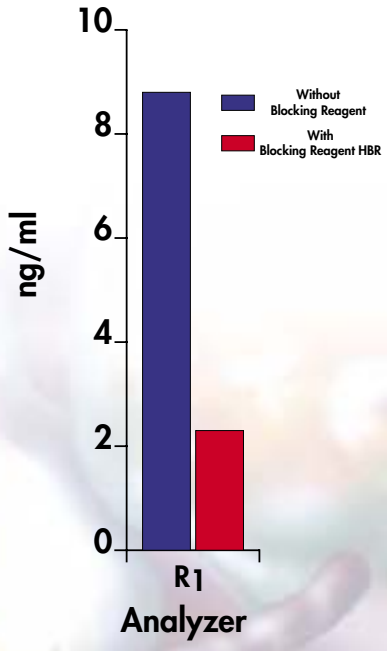
Prolactin



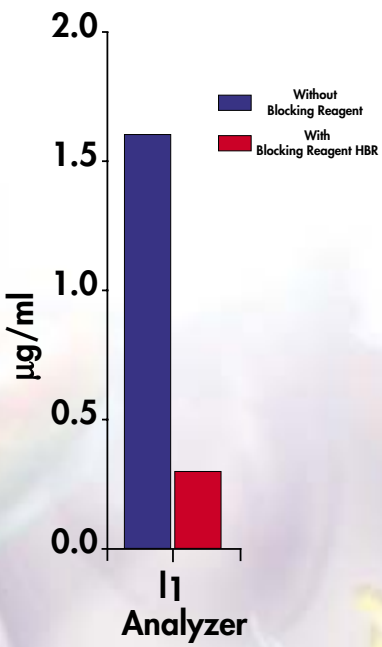
TG



TG



CKMB



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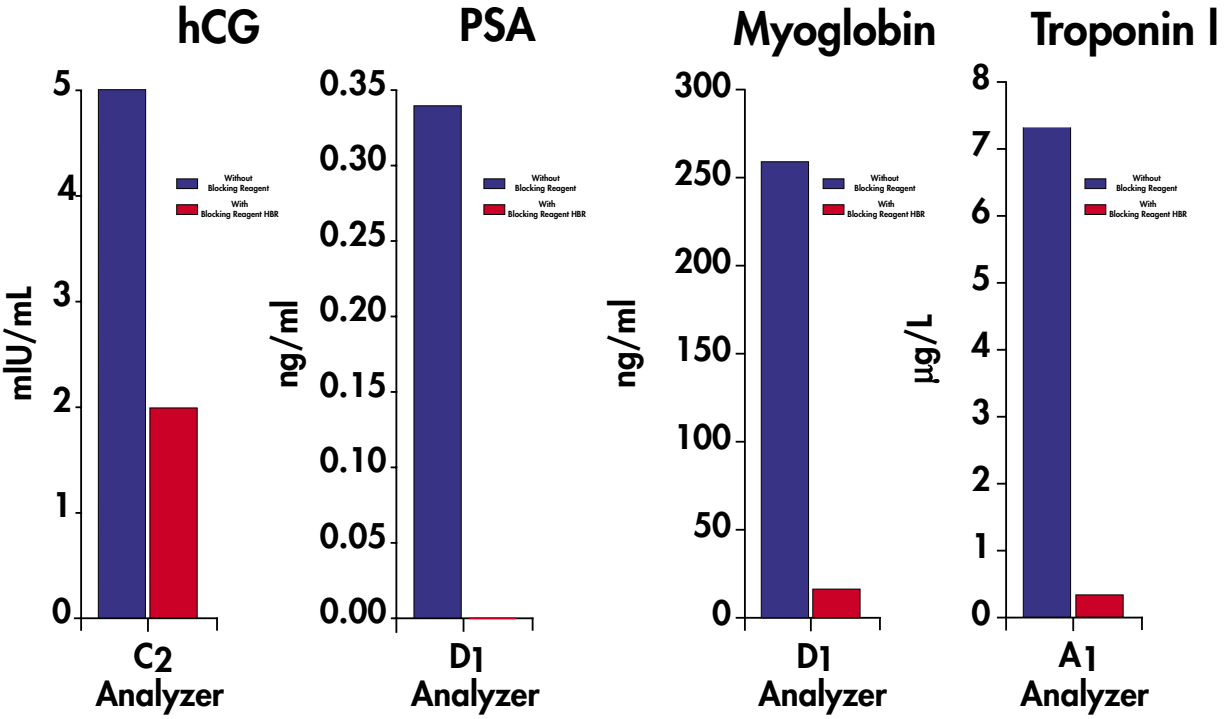
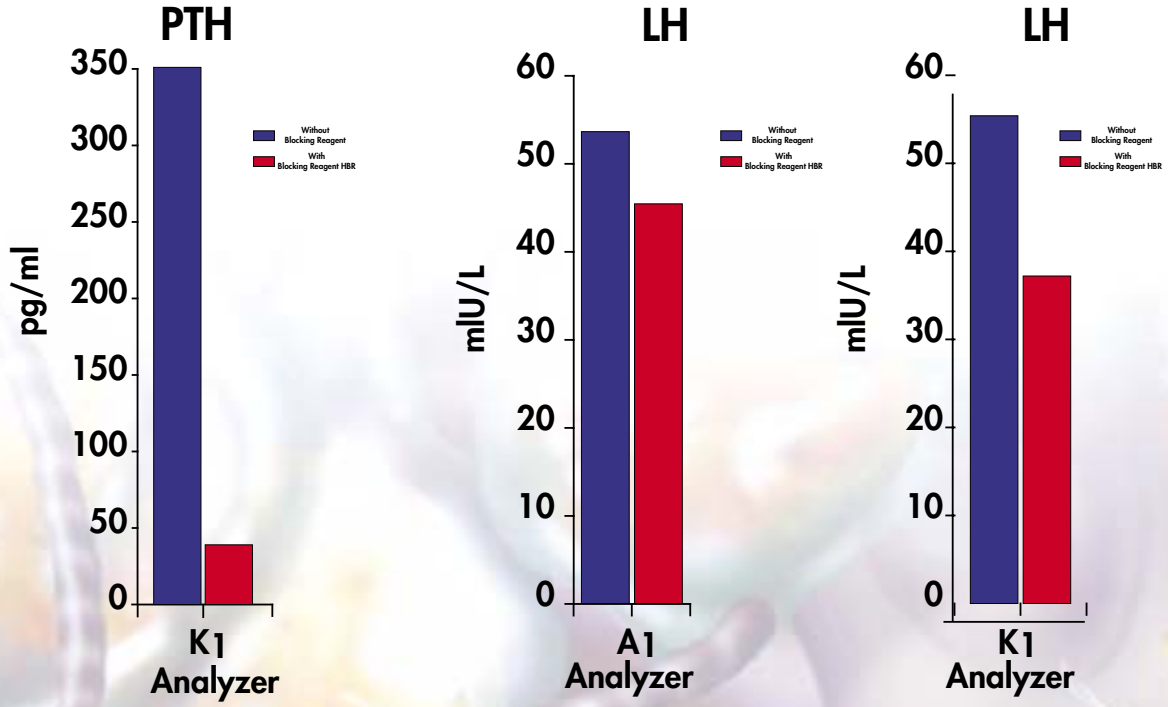
continued

False Positive Studies in Immunoassay Systems using one Heterophilic Antibody Positive Sample

Donor #2





Note: Plateau Values were not established for the above blockers. If more HBR were used, values would decrease further.

In an independent study of patients receiving CA125 immunotherapy, **75%** were found to be **false positive.**

CA 125 and CEA false positives from patient samples in one day in a clinical lab

	Lab Findings (Based on patient samples)	vs	Claims (Probably based on normals)	Accuracy Improvement-HBR Before HBR	After HBR
CA 125™	9.2%	vs	1% - 2%	91% Accurate or 10% false positives	→ 98.2%* Accurate or 1.8% false positives
CEA	22%	vs	1% - 2%	78% Accurate or 22% false positives	→ 98.5% Accurate or 1.5% false positives

Scantibodies has found ~10x higher false positive prevalence in patients versus normals

False positive troponin I measurements
Dr. Schifman's results

"Between-Assay variation in false positive troponin-I measurements in patients on renal dialysis or with positive rheumatoid factor". Schifman, R. B., James, S.H. Sadrzaden, S.M.H., Rose, A., Dick, S., Departments of Pathology and Internal Medicine, Veterans Affairs Medical Center and University of Arizona Health Sciences Center, Tucson, AZ , Published: *Clinical Chemistry 45, No. 6, Supplemental, 1999. (Abstract 516)*

Chart 1

Results

rheumatoid factor specimens

	% false positives*	mean concentration of false positives
Company A	3.1%	2.0 ng/ml
Company B	1.6%	3.8 ng/ml
Company C	9.4%	11.7 ng/ml

*interpreted as false positives by clinical history

Chart 2

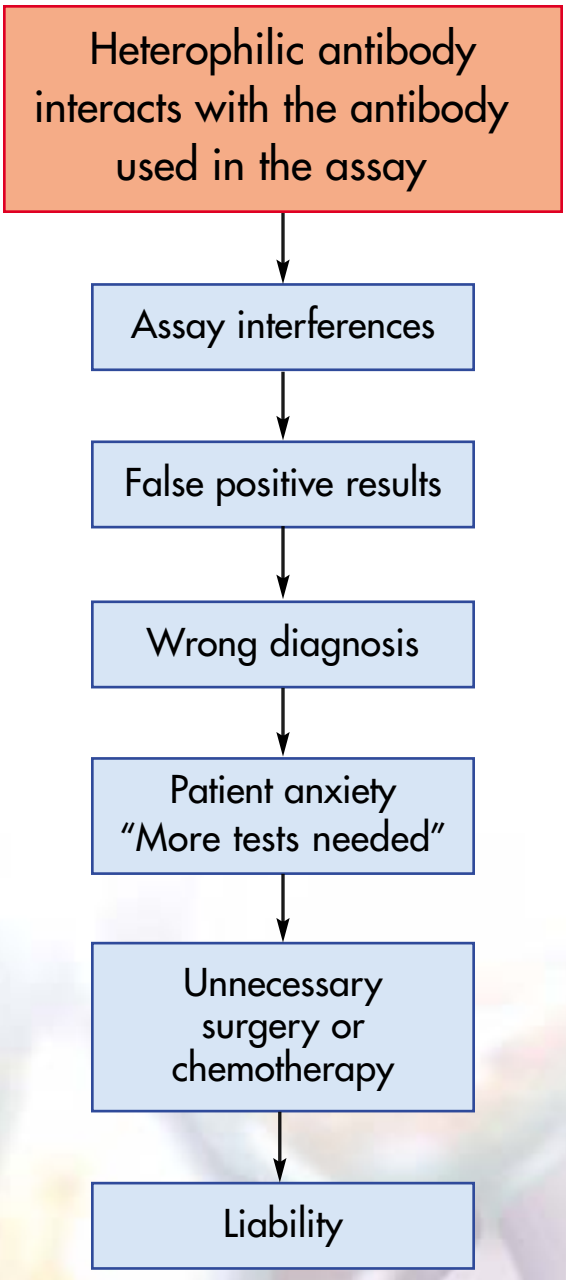
Results

dialysis patient specimens

	% false positives*	mean concentration of false positives
Company A	1.0%	2.6 ng/ml
Company B	0.7%	1.5 ng/ml
Company C	1.4%	2.8 ng/ml
Company D	4.2%	0.44 ng/ml

*interpreted as false positives by clinical history

How the presence of heterophilic antibodies in a patient's serum can lead to serious consequences.



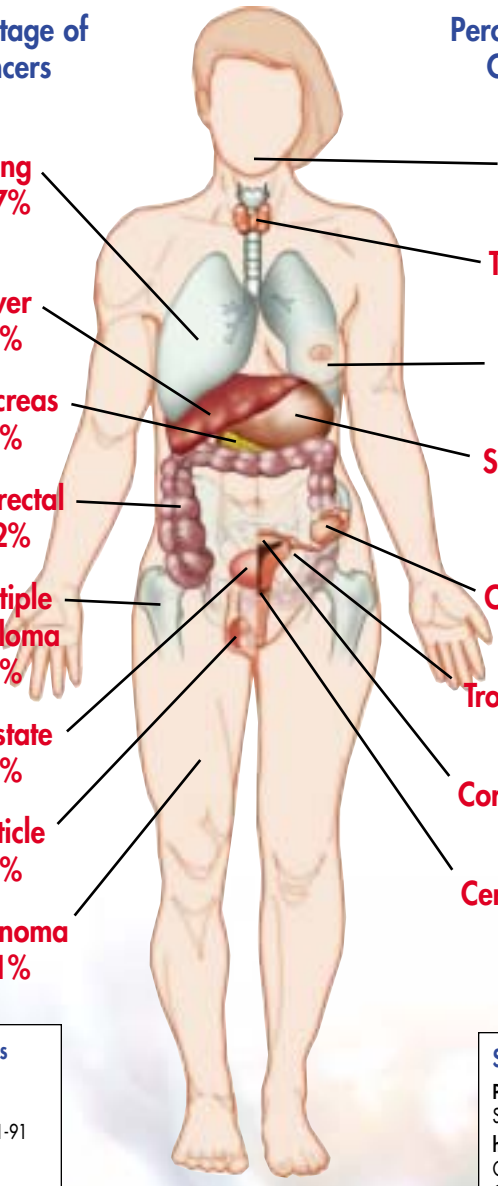
Consequences of Reporting False Positives in Cancer

One out of four people will have cancer in his/her lifetime. The following shows the frequency (%) of different types of cancer as part of the total number of cancers and the consequences of a False Positive test result.

If used exclusively by the physician for diagnosis, a False Positive test result may lead to unnecessary treatment such as:

- SURGICAL REMOVALS
- CHEMOTHERAPY
- RADIATION/RADIOLOGICAL THERAPY

Tests which could yield False Positives	Percentage of Cancers	Percentage of Cancers	Tests which could yield False Positives
ACTH, PTH, SCC, NSE, CEA, CYFRA, Prolactin, Renin	Lung 27%	ENT 2%	SCC, CEA
Somatomedin-C, AFP, CA19-9, CEA	Liver 2%	Thyroid 1%	hCT, TG, TPA, CEA, NSE
CA19-9, Gastrin	Pancreas 4%	Breast 18%	CA15-3, CEA
CA19-9, Gastrin, CEA	Colorectal 12%	Stomach 12%	CA72-4, CA19-9, CEA, Gastrin
ß2-microglobulin	Multiple Myeloma 1%	Ovarian 7%	CA125, CA19-9, CA72-4
		Trophoblast < 1%	hCG
PSA, PAP	Prostate 3%	Corpus Uteri 4%	SCC, CEA, CA125
SCC, AFP, hCG	Testicle 2%	Cervix Uteri 4%	SCC, CEA, CA125
5-5-Cysteinyl dopa	Melanoma <1%		



Frequency (%) of kinds of cancer (from Becker et.al. Krebsatlas der Bundesrepublik.1984).

Some prominent False Positive References

CEA
Morton BA, et al. Arch Surg 1988; 31:1242-6
Kuroki M, et.al. J Immunol Methods 1995; 180:81-91
Kricka LJ, et al. Clin Chem 1990; 36:892-4

PROLACTIN
Dericks-Tan JS, et al. Klin Wochenschr 1984; 62:265-73
Hellthalar G, et al. Geburtsh Frauenheilkd 1995; 55:M55-6

AFP
Bussar-Maatz R, et al. *Urologe A* 1993; 32:177-82

Some prominent False Positive References

PSA
Stowell LI, et al. *Forensic Sci Int* 1991; 50:125-38

hCG
Cole LA, Gyneco Onco 1998; 71:325-9
Cole LA, Clin Chem 1999: 45:313-4
Vladutiu AO, et al. JAMA 1982; 248:2489-90

CA125
Turpeinen U, et al. Clin Chem 1995; 41:1667-9
Turpeinen U, et al. Clin Chem 1990; 36:1333-8
Boerman OC , et al. Clin Chem 1990; 36:888-91
Reinsberg J, et al. Clin Chem 1990; 36:164-7

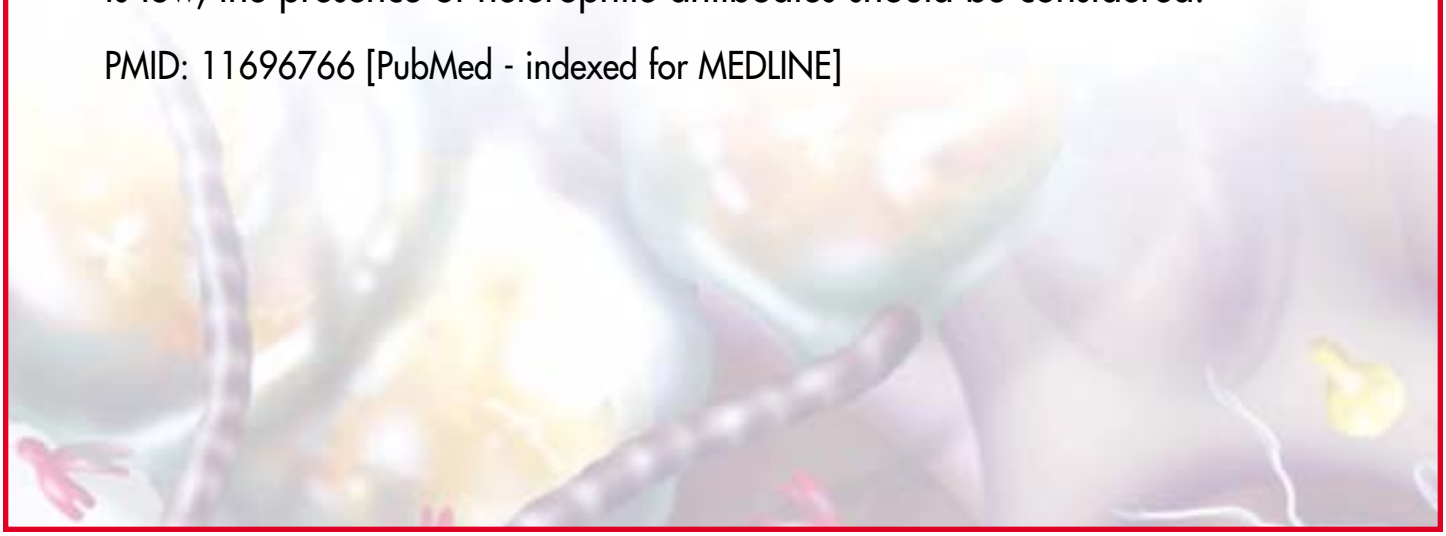
Serum heterophile antibodies interfere with prostate specific antigen test and *result in over treatment* in a patient with prostate cancer.

Morgan BR, Tarter TH.

Department of Pathology, Carle Clinic Association and University of Illinois School of Medicine at Urbana-Champaign, USA.

PURPOSE: We evaluated how naturally occurring heterophile antibodies in patient serum interfered with prostate specific antigen (PSA) immunoassay, resulting in over treatment for prostate cancer. MATERIALS AND METHODS: Serum samples were treated with heterophilic blocking reagent (Scantibodies Laboratory, Inc., Santee, California). Treated and untreated samples were tested by the Medics (Tosoh, Foster City, California) Tandem-R (Beckman-Coulter Inc., Chaska, Minnesota) and Elecsys (Roche Molecular Biochemical, Indianapolis, Indiana) PSA assays. Heterophile antibodies were measured directly in treated and untreated samples by the human anti-mouse antibody immunoradiometric assay and heterophilic antibody identification enzyme immunoassay (Scantibodies Laboratories, Inc.). RESULTS: Human anti-mouse Ig heterophile antibodies in patient serum caused false-positive PSA test findings after radical prostatectomy, **resulting in over treatment** for presumed disease recurrence. CONCLUSIONS: If PSA is detectable after radical prostatectomy and the likelihood of incomplete resection or systemic disease is low, the presence of heterophile antibodies should be considered.

PMID: 11696766 [PubMed - indexed for MEDLINE]



Two cases of false troponin I increase in patients with heterophile antibodies

[Article in Italian]

Cassin M, Cappelletti P, Rubin D, Zaninotto M, Macor F, Nicolosi GL.

U.O. di Cardiologia A.O. Santa Maria degli Angeli Via Montereale, 24 33170 Pordenone. mat54@iol.it

Cardiac troponin T and I are highly sensitive and specific biochemical markers for the detection of myocardial damage and they are now considered the preferred markers for the diagnosis of myocardial infarction. Despite this, in some cases elevations in the serum levels of cardiac troponin T and I are not associated with a final diagnosis of cardiac necrosis. These false-positive results are to be related to different interferences in immunometric assays. We report 2 cases of false-positive troponin I results due to heterophilic antibodies. Two women admitted to the Emergency Department with acute chest pain persistently showed, in serial blood samples, elevated and constant values of troponin I serum levels. The results were confirmed as being false positives by treatment of the samples with heterophilic blocking reagent (Scantibodies Laboratory, Santee, CA, USA). Coronary artery disease was excluded at coronary angiography and at stress testing in the first case and at stress myocardial perfusion imaging in the second case. In clinical practice, in case of persistently elevated but constant values of cardiac troponin without the time interval of release characteristic of acute syndromes, it is important to bear in mind the possible occurrence of false-positive cardiac troponin results due to the presence of heterophilic antibodies.

PMID: 11926033 [PubMed - indexed for MEDLINE]

Consequences of a false positive Troponin I result (for myocardial infarction)

Clinical History:

- A patient with chest pain
- On 11-20-99, R.G. (87058), a 50 year-old gentlemen, was out deer hunting, and after walking two miles, he returned to his truck. He suddenly developed a sharp substernal discomfort … it felt like a sledge hammer hitting his chest—similar to the myocardial infarction he had in 1997, though much less severe. After two sprays of nitroglycerin, the pain decreased. Since 11-20-99, he has had chest discomfort six times.
- On 11-29-99, while he was getting dressed, he again developed substernal chest discomfort. At Victory Medical Center, (Stanley, WI), he was treated with nitroglycerin and IV heparin. His Troponin I was noted to be 23.3 ng/ml (normal level is n<2 ng/ml) by a widely used analyzer. Cardiac catheterization was done. No significant CAD (Cardiac Arterial Disease) noted. Aortic stenosis was identified and he was managed medically without chest complaints at the time of discharge.
- On 12-27-99, when awakened in the morning, he experienced the onset of chest pain. He went to work and the chest pain intensified.
- He went to the ER and was started on IV nitroglycerin. A 12 lead EKG showed a left bundle branch block unchanged from previously (below).
- Repeated coronary angiography-normal.
  Consulted lab and referred to Infectious Medicine.
  Sample treated with HBT showed a cTPI of 0.1 ng/ml.
  The untreated sample was also analyzed on another analyzer:
  cTPI was <0.1 ng/ml.

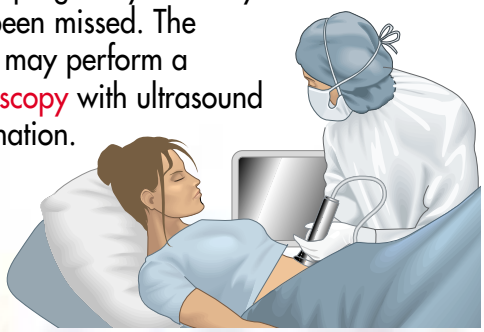
		cTPI	CK-MB	CK
14:22	(12-27)	30.1	1.4	353
23:08	(12-27)	28.6	0.8	1192
6:10	(12-28)	28.2	0.4	1005

CRP 12.1ng/dl (normal level is <1.5 ng/dl)

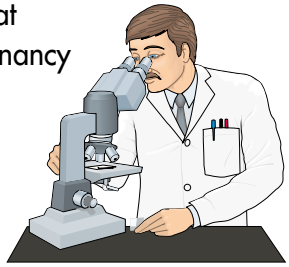
How an hCG (pregnancy test) false positive result can cause the problems cited in the 1999 Clinical Chemistry article by Dr. Lawrence Cole of Yale University

1. First, a female patient goes into the hospital to receive an X-ray or MRI and hospital personnel routinely perform an hCG pregnancy test to ensure no danger exists to a baby. When the result comes back as positive, the doctor suspects pregnancy and asks the patient to return in 2 weeks for another test.

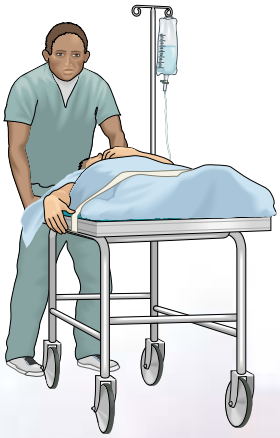
2. The patient returns and the hCG level (which is really a false positive) has not risen. The doctor may suspect an extra-uterine or ectopic pregnancy and typically will perform a trans-vaginal ultrasound, which after 6 weeks should reveal a fetal sac. When no fetal sac is detected, the doctor may suspect more strongly an ectopic pregnancy. He may place the patient on methotrexate to stop an ectopic pregnancy that may have been missed. The doctor may perform a laparoscopy with ultrasound examination.



3. The doctor may now move to perform a dilation and curettage (D & C), and send the tissue to the pathologist who will look for molar tissue or pregnancy tissue. When the pathologist reports that neither molar or pregnancy tissue was found, the doctor may now suspect the rare occurrence of chorio-carcinoma.



4. If the doctor confirms that the patient has had a previous pregnancy, the doctor may proceed with treatment for post-gestational choriocarcinoma which may include: methotrexate and adriomycin treatments, hysterectomy, oophorectomy, and removal of other suspected tissues that may be involved in a chorio-carcinoma. If the hCG level continues to be elevated, the physician may put the patient on EMACO chemotherapy which may result in coma and type 1 diabetes.



"False-Positive hCG Assay Results Leading to Unnecessary Surgery and Chemotherapy and Needless Occurrences of Diabetes and Coma,"

Clinical Chemistry, 45, No. 2, 1999, pages 313-314, Laurence A. Cole, Kirsi M. Rinne, Shohreh Shahabi, Aziza Omrani, Department of Obstetrics and Gynecology, Yale University, New Haven, CT 06520

Table 1. Summary of clinical findings and laboratory data.

	Patient					
	i[a]	ii[a]	iii[a]	iv	v	vi
Age, years	24	26	36	36	22	28
hCG test	Abbott AxSym hCGβ	Abbott AxSym hCGβ	Abbott AxSym hCGβ	Abbott AxSym hCGβ	Abbott IMX hCGβ	Bayer Immuno-1 total hCG
Initial hCG, IU/L	117	52	191	385	205	69
Range of hCG, IU/L[b]	45–135	22–89	145–351	78–451	5–205	48–74
Laparoscopy	✓	✓	✓	✓	✓	✓
Dilation and curettage	✓	✓	✓	✓	✓	✓
Oophorectomy			✓			
Hysterectomy		✓		✓		
Methotrexate chemotherapy	✓	✓	✓	✓		
EMACO chemotherapy				✓		
Type 1 diabetes and coma				✓		

[a] Patients i, ii, and iii are described in more detail in Cole *(3)*.

[b] Range of hCG concentrations in the 3–11 months (depending on case) after initial detection.

Is a False Positive Result in an Infectious Disease Assay less serious?

CAP Today April, 2003

"I have participated in many discussions in which comments were made that getting a false-positive test for hepatitis C isn't serious, or isn't as serious as getting one for HIV," Dr. Alter reports. "That's appalling. I think it's appalling that anyone would have that attitude toward giving a patient a false-positive result, and then subjecting them to not only the psychological stress but also the expense of additional evaluation when it isn't necessary."

Dr. Alter
Associate Director for
Science Center for
Disease Control
Division of Viral Hepatitis

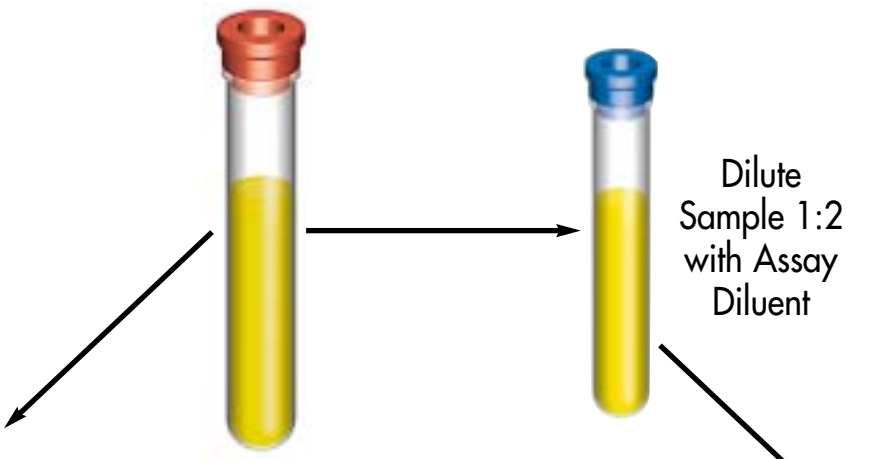
Any one of the
false positive test methods
is able to identify a
false positive

but

a false positive
may not necessarily
be detected by any one
of the false positive
test methods

Three Methods to Identify a False Positive

1. Non-Linear dilution method



Assay Undiluted Sample

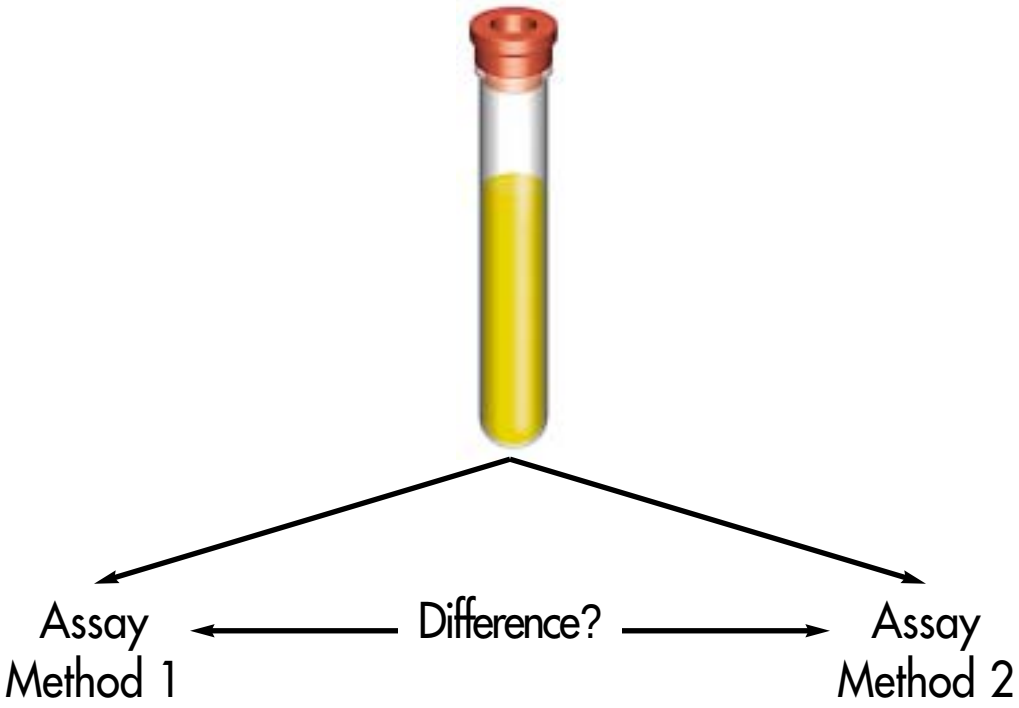
is Diluted Sample

Assay Value 40%-60% of Undiluted Sample Assay Value?

Assay Diluted Sample

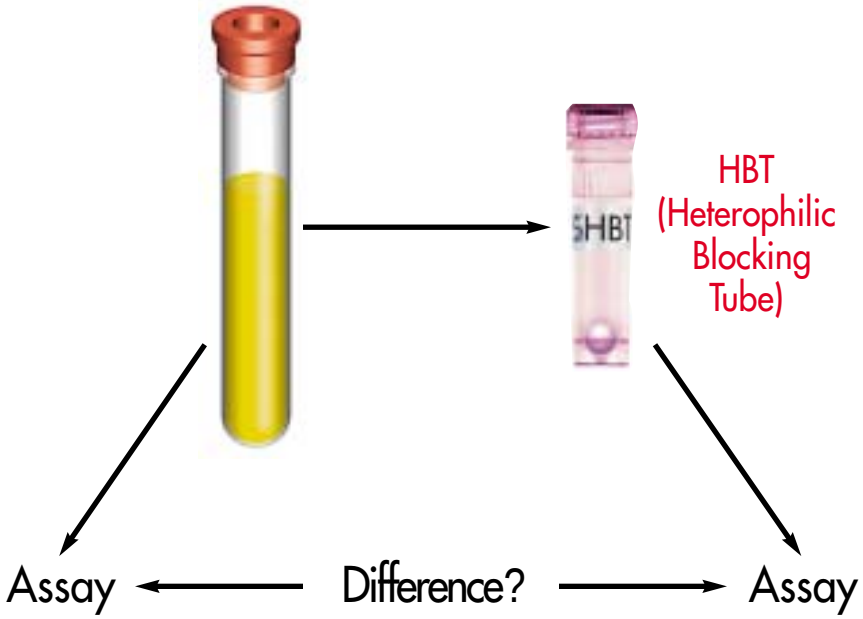
Three Methods to Identify a False Positive

2. Alternate assay method

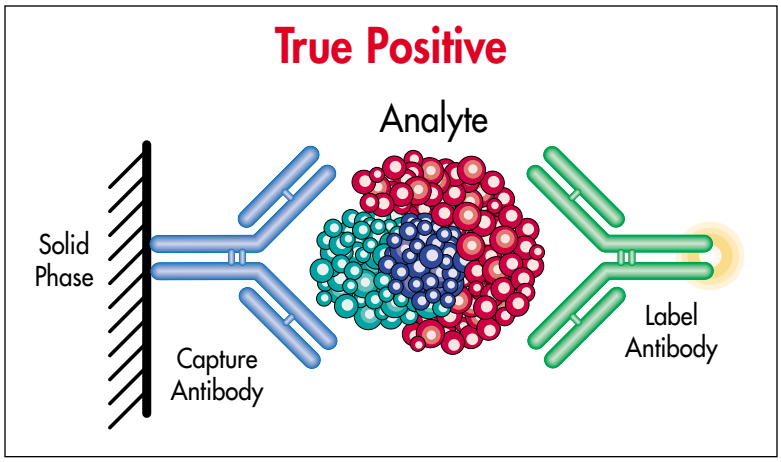


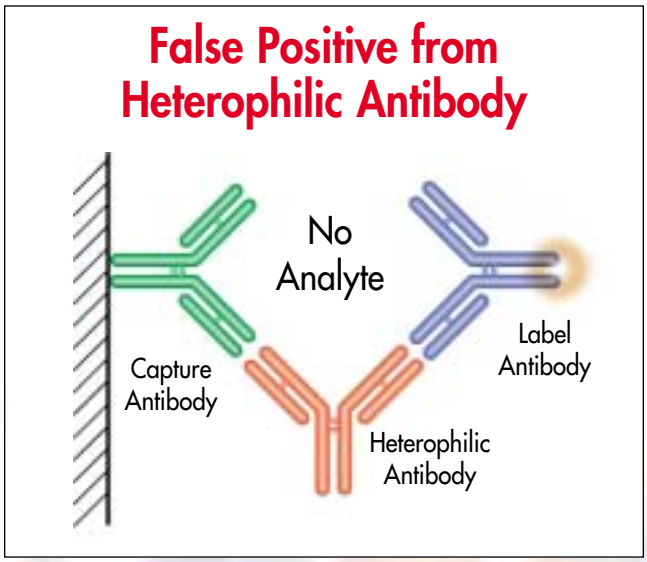
Three Methods to Identify a False Positive

3. Blocker method

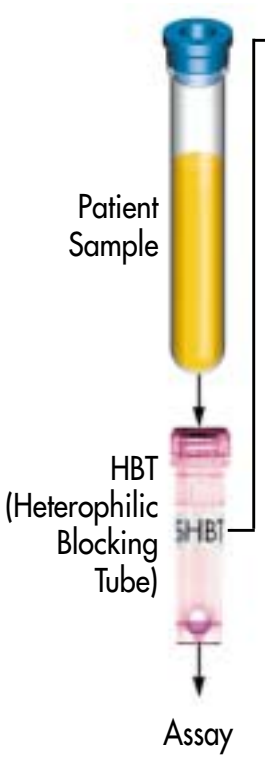


How do the heterophilic antibody interactions result in false positives in solid phase-based sandwich immunoassays?



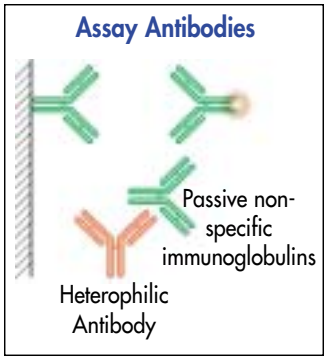


How to reduce assay susceptibility to false positives



Contains

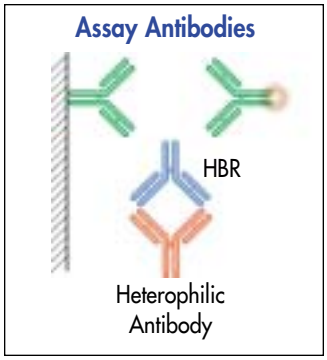
Passive Heterophilic Antibody Blockers



Passive

Immobilized IgG
Non-immune serum
Irrelevant monoclonals
Antibody fragments
Polymerized IgG

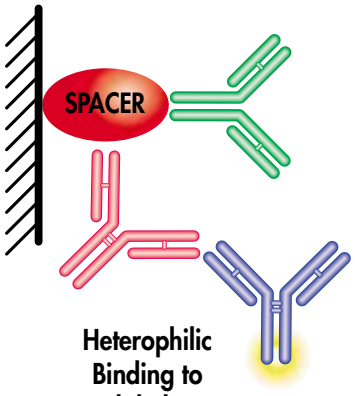
Active Heterophilic Blocking Reagents (HBR)



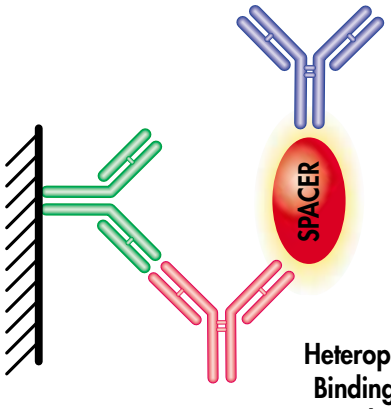
Active

HBR

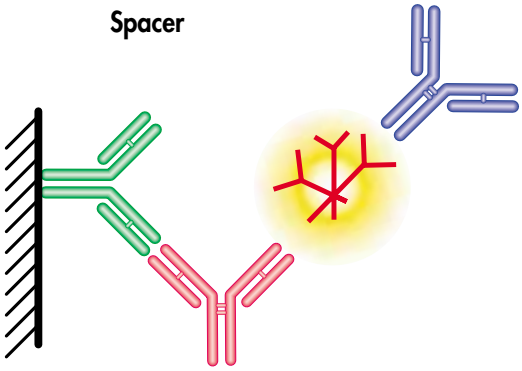
Heterophilic antibodies may attach to a variety of assay antibodies binding sites



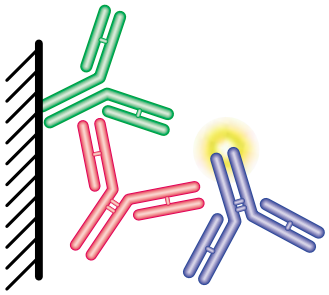
Heterophilic Binding to Solid Phase Spacer



Heterophilic Binding to Label Spacer

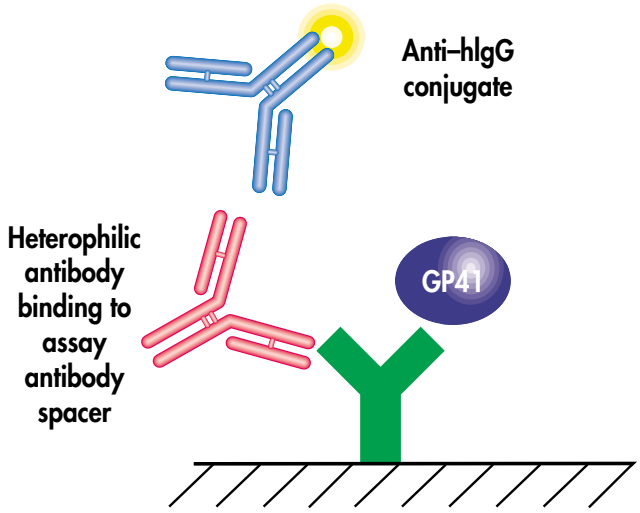


Heterophilic Binding to Aggregated Label

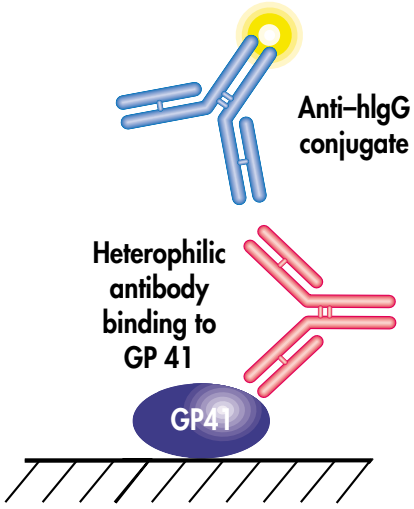


Heterophilic Binding to F_C of Assay Antibodies

How do the heterophilic antibody interactions result in false positives in a serological assay?

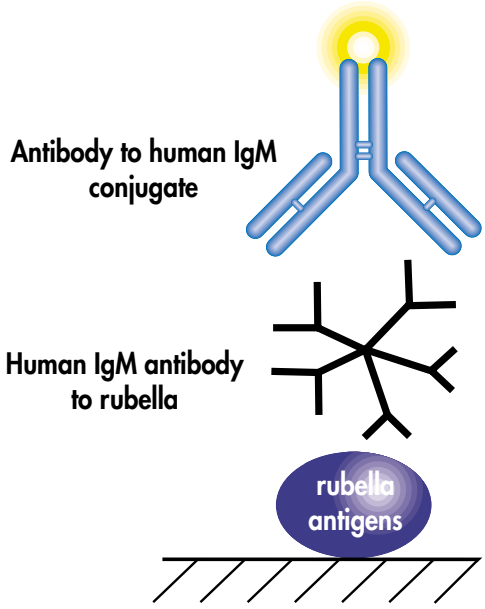


A. False positive in an antigen capture assay

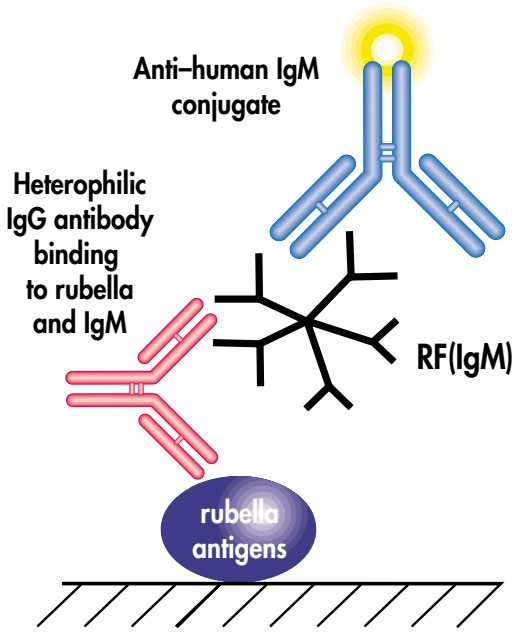


B. False positive in direct antigen coating assay

How do the heterophilic antibody interactions result in false positives in the detection of IgM antibodies?

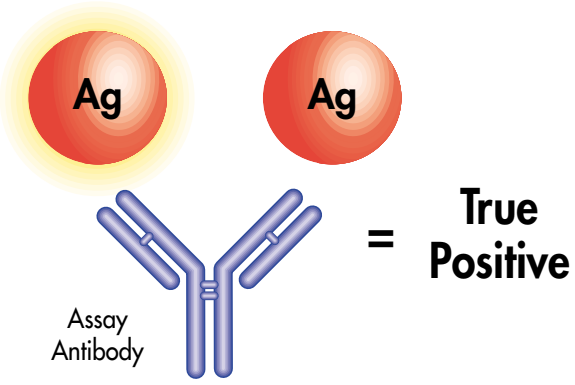


True positive

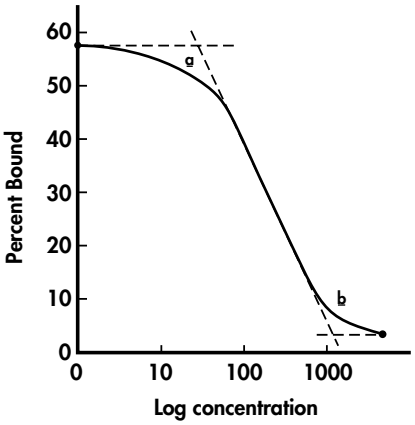


False positive for IgM rubella antibody

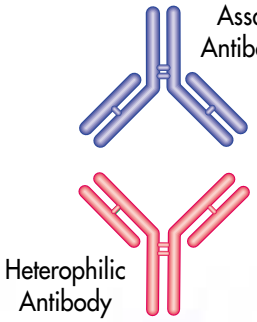
How do the heterophilic antibody interactions result in false positives in a competitive binding assay format?



= True Positive



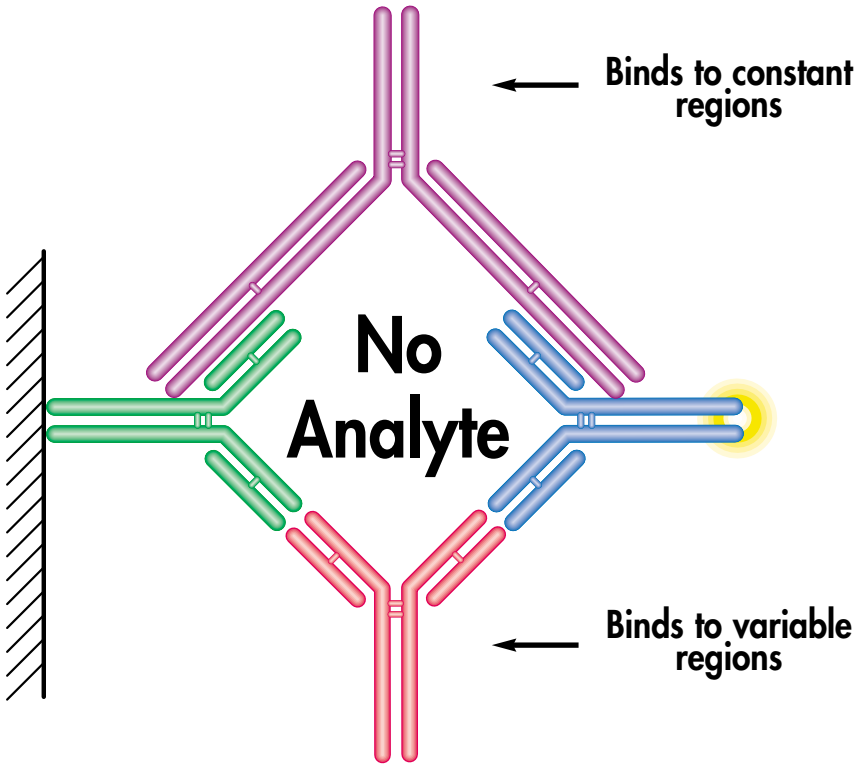
False positive from heterophilic antibody binding to the antibody used in the assay



= False Positive

Two types of Heterophilic Antibodies

I. Anti-Isotypic



II. Idiotypic (anti-hypervariable)

Interference Prevention

I. In the Patient

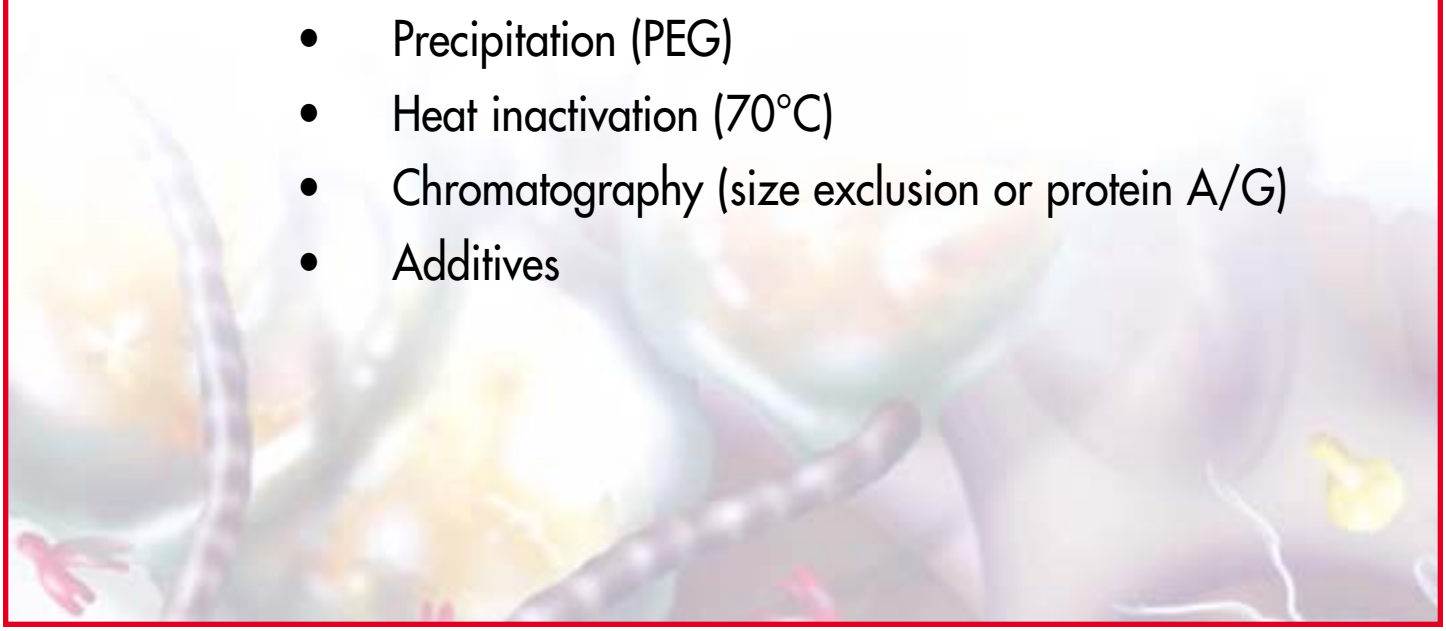
- Immunosuppressant therapy
  (Cyclosporine A)
- Antibody fragment
- Humanized and chymeric antibodies
  (mouse CDR and human framework)
- Pegylation
  (stealth fighter)

II. Assay Redesign

- F(AB') conjugate
- Use chymeric antibodies in the assay
  (Roche patent)
- Chicken antibodies
  (no cross-reactivity but no MAb's)

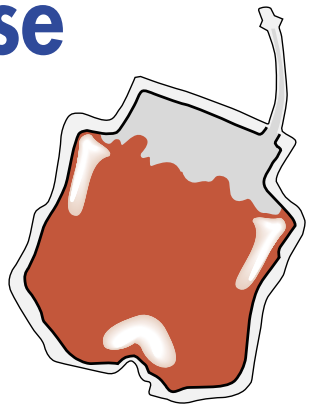
III. Sample Pretreatment

- Precipitation (PEG)
- Heat inactivation (70°C)
- Chromatography (size exclusion or protein A/G)
- Additives



Infectious Disease

Hepatitis



- Situation:

  In a study conducted by the Finnish Red Cross

HBR fixed almost half of their false positives.

- Consequences:

  In 1999, their 673 false positive results cost them over $500,000.

- Solution:

  HBR used with the HBsAg Assay would save about $250,000.

Finnish Red Cross
Blood Transfusion Service

What the Manufacturer can do proactively to reduce the reporting and consequences of false positives

FDA

Patient → Doctor → Lab ← Manufacturer → Regulatory Agency

Clinical Use ✕ ✕ "Clinical Use" ← Clinical Trials & Approvals → Approves Product for Market

("This assay is intended to be used to test for...")

Clinical Evidence ✕ ✕ "Clinical Evidence Disclaimer" ← Product Shortcomings → Approves P.I.

"If clinical evidence does not correlate with result, consider false result."

The Lab can

- Through literature, foresee how test could be used and the false positive consequences.
- Communicate "Clinical Use Restrictions and Clinical Evidence Disclaimers" to doctors.
- Recognize that a doctor using HCG "on-label" will inevitably use it for choriocarcinoma ("off-label").

The Manufacturer can

- Through literature, foresee how test could be used and the false positive consequences; *e.g., recognize that a doctor using HCG "on-label" will inevitably use it for choriocarcinoma ("off-label").*
- Add to P.I. "Clinical Use Restrictions" (i.e. "This test not to be used for cancer diagnosis.")
- Recognize the ineffectiveness of the P.I. and communicate "Clinical Use Restrictions and Clinical Evidence Disclaimers" to doctors. (Either directly to doctors or in cooperation with labs).
- Improve the product with problematic samples and blockers.

The RA can

- Through literature, foresee how test could be used and the false positive consequences.
- Require specific "Clinical NON-use claims."
- Require effective communication of P.I. "Clinical Use/Non-Use Restrictions" and "Clinical Evidence Disclaimers" to doctors.

"Most physicians never read the package insert".
(Larry Kricka)

II.
Developing a blocker formulation

I.
Identifying and purchasing 100 ml. of a minimum of 5 false positive samples

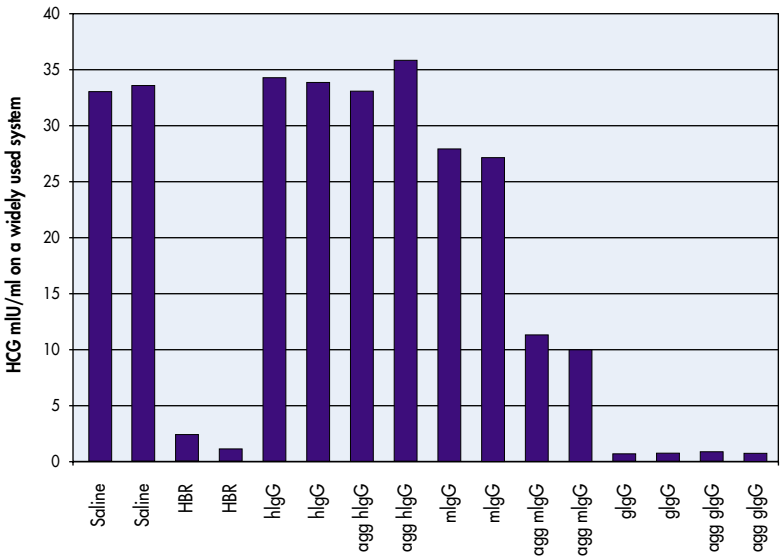
(Screening program)

How to develop a blocker formulation

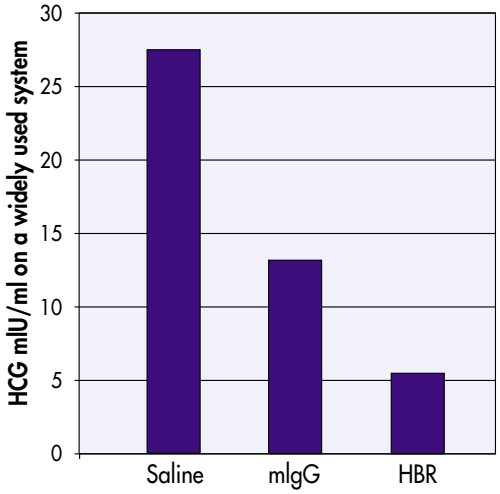
- For each false positive sample (panel), evaluate Individual Action of each blocker (determine plateau value)
- Combine all plateau values for all effective blockers (ADBK) into one blocker formulation and retest each false positive sample
- Back off individually on each blocker component and retest all samples, looking for the Synergistic Action to optimize blocker formulation (reduce costs)

Three false positive samples for β-hCG on a widely used system

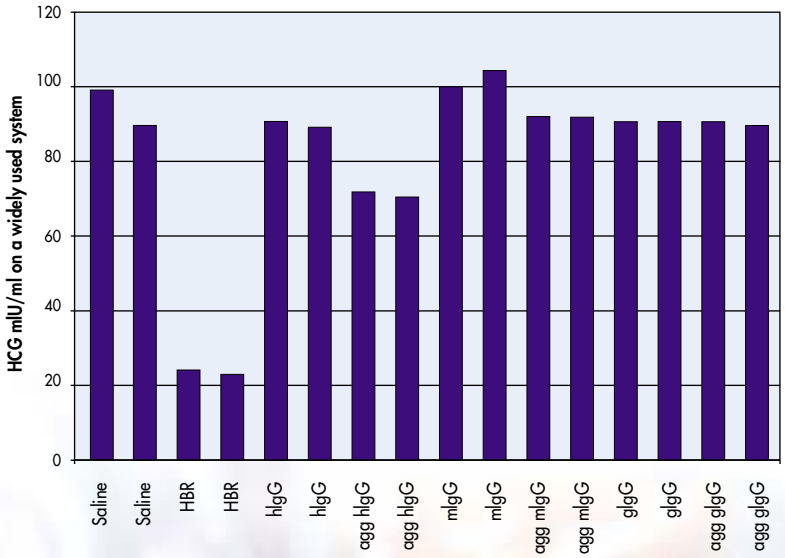
Sample No. 19107-9696



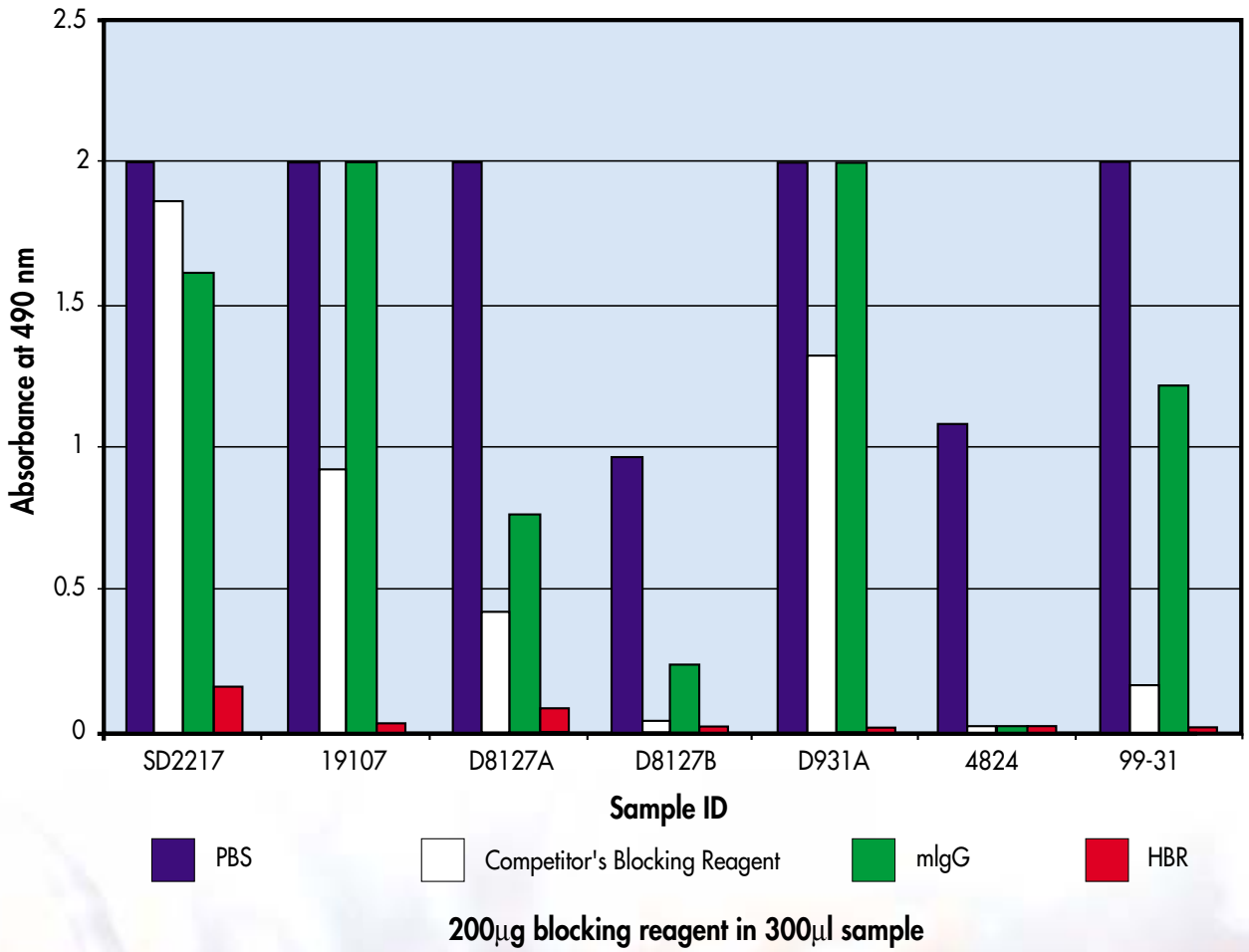
Sample No. 1587058



Sample No. SD2217-1

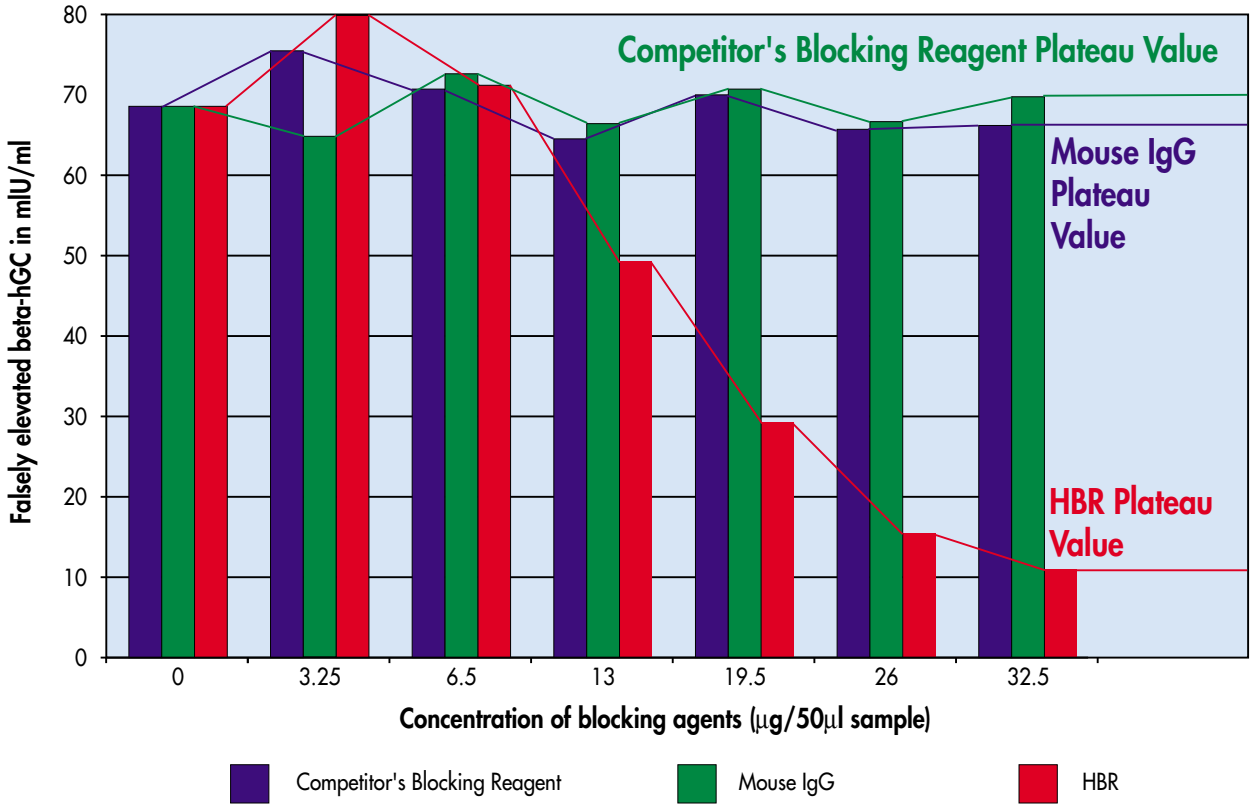


Efficiency of HBR-1 vs mIgG and competitor's blocking reagent in reducing interferences using a dual mouse monoclonal sandwich assay for seven false positive samples



200µg blocking reagent in 300µl sample

Plateau value of HBR in comparison to mouse IgG and competitor's blocking reagent in reducing false positive interference in a widely used β-hCG assay



"What you really need is a nice set of samples that represent the range of anti-animal antibody interferences. Then perhaps another set to help you evaluate assays that you're either using or were developing to show that your optimization studies to remove interferences have worked."

– Larry Kricka, D. Phil.
Professor or Pathology
University of Pennsylvania
Medical Center

The Effect of HBT on hCG Immunoassay Results

Case	Reference Service hCG test (IU/L)	Physician's Laboratory hCG test (IU/L)	hCG Reference Service hCG test + HBT (IU/L)
1	15	9.4	<1.1
2	60	<1.1	<1.1
3	97	18	<1.1
4	114	3.6	<1.1
5	402	<1.1	<1.1
6	80	>110	<1.1
7	>600	3.3	<1.1
8	93	11	<1.1
9	68	13	6.5
10	110	14	<1.1
11	133	5.1	<1.1
12	220	4.8	2.2

Dartmouth and Harvard report false positive heart attack test

False Increase of Cardiac Troponin I with Heterophilic Antibodies, *Thomas F. Fitzmaurice,[1] Charles Brown,[1] Nader Rifai,[2] Alan H.B. Wu,[3] and Kiang-Teck J. Yeo[1]* (Department of Pathology, Dartmouth-Hitchcock Medical Center and Dartmouth Medical School, [1] Medical Center Drive, Lebanon, NH 03756; 2Department of Laboratory Medicine, Children's Hospital & Harvard Medical School, Boston, MA 02115, and [3] Clinical Chemistry Laboratory, Hartford Hospital, Hartford, CT 06102.

Table 1. Comparison of cTnI values for a plasma pool from a patient with suspected HA and a positive-control pool from a patient with confirmed acute myocardial infarction.

	HA sample		Positive control	
Assay	Untreated	Treated[a]	Untreated	Treated[a]
Axsym cTnI, μg/L	84.0	1.5	147	135
Opus cTnI, μg/L	<0.5	<0.5	43.1	34.6
Immuno-1 cTnI, μg/L	0.0	0.0	22.8	21.7
Dade Dimension cTnI, μg/L	0.12	0.08	29.5	31.4
Roche/BMC cTnT, μg/L	0.02	0.02	10.2	10.3

[a] Refers to treatment with heterophilic blocking agent.

reference values for this sample. The sample from the positive-control individual showed a small decrease on HBR treatment, which has been noted previously and attributed to the blocking agent (*8*). When samples from this patient were assayed for total CK, CK-MB, and cTnI in the presence or absence of HBR, only the AxSYM cTnI assay was completely blocked by the HBR. In addition, there was no clear peak observed for serial cTnI, suggesting an absence of an evolving myocardial injury (Fig. 1, panels A–C). The increase in total CK and CK-MB is consistent with skeletal muscle regeneration after surgery. The results of these studies suggested to us that HAs were present in the patient's plasma.

If HAs are the cause of the falsely increased cTnI concentration, we questioned why the AxSYM CK-MB assay, which is also a sandwich assay based on a similar microparticle enzyme immunoassay format, was not affected. Both assays utilize a mouse monoclonal antibody as the primary, or "capture", antibody and a goat polyclonal antibody as the secondary, or "labeled", antibody. On further examination of reagent composition, it was noted that, although the mouse monoclonal anti-troponin I reagent contains mouse and goat proteins, the reagent containing the goat anti-troponin I conjugate antibody contains bovine and fish stabilizers. In the AxSYM cTnI procedure, the sample is first incubated with the monoclonal antibody reagent in the reaction vessel before being transferred to the matrix cell and washed. The conjugate antibody reagent is then added, and a second incubation takes place.

Knowing this sequence of events, we propose the following: Multispecific mouse/goat antibodies present in the patient's serum at sufficient titer to overcome the effect of the "blocking" proteins bind to the mouse monoclonal anti-cTnI during the first incubation. An aliquot of this is added to the matrix cell and washed, removing all unbound proteins, including the blocking proteins. The conjugate antibody is then added, in the absence of any proteins capable of blocking the binding of the HAs to the goat immunoglobulin. The mouse anti-cTnI and goat anti-cTnI cross-link, producing a false increase of the measured cTnI concentration.

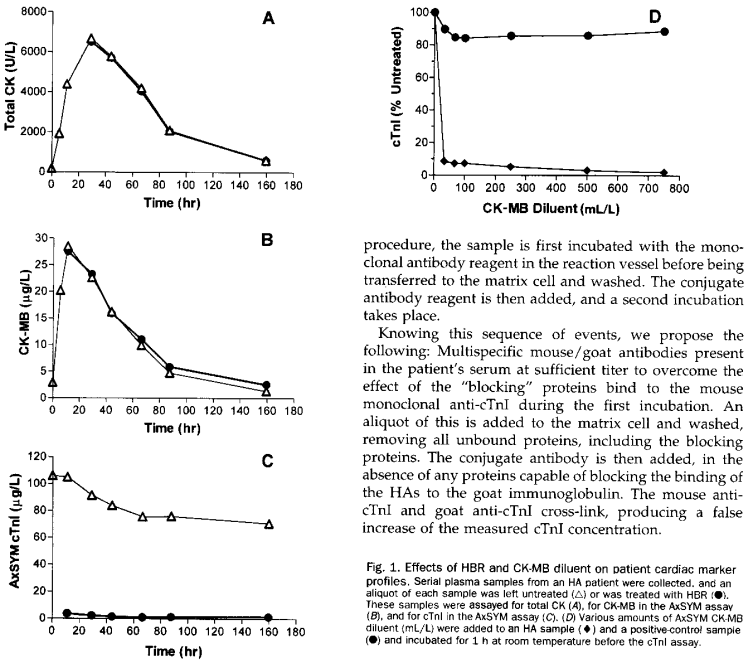


Fig. 1. Effects of HBR and CK-MB diluent on patient cardiac marker profiles. Serial plasma samples from an HA patient were collected, and an aliquot of each sample was left untreated (△) or was treated with HBR (●). These samples were assayed for total CK (*A*), for CK-MB in the AxSYM assay (*B*), and for cTnI in the AxSYM assay (*C*). (*D*) Various amounts of AxSYM CK-MB diluent (mL/L) were added to an HA sample (◆) and a positive-control sample (●) and incubated for 1 h at room temperature before the cTnI assay.

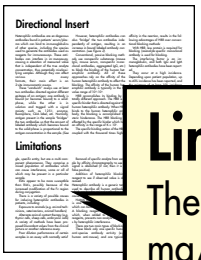
attributed to the blocking agent (*8*). When samples from this patient were assayed for total CK, CK-MB, and cTnI in the presence or absence of HBR, only the AxSYM cTnI assay was completely blocked by the HBR. In addition,

The FDA Says:

"The Food and Drug Administration (FDA) has recognized the importance of anti-animal antibodies such as HAMA. In its "review criteria for assessment" documents, the FDA recommends that labeling (e.g., package insert) of an *in vitro* diagnostic device list as a limitation the following: "As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample" (19). In more recent documents, the FDA recommends the following: "If the assay kit employs mouse monoclonal antibodies, include a warning that specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA) and may show either falsely elevated or depressed values when tested." (20).

Ref: Kricka, Larry J., "Human Anti-Animal Antibody Interferences in Immunological Assays," *Clin Chem 45:7, 942-956 (1999)*

Anti-HAV Directional Insert


Directional Insert

Limitations

Limitations – interference

The assay is unaffected by icterus (bilirubin < 855 umol/l or < 50 mg/dl), hemolysis (Hb < 0.745 mmol/l or < 1.2 g/dl), lipemia (Intralipid < 1000 mg/dl), and biotin < 50 ng/ml.

Criterion: Recovery within plus/minus 10% of initial value.

In patients receiving therapy with high biotin doses (> 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1600 U/ml.

In vitro tests were performed on 18 commonly used pharmaceuticals.

No interference with the assay was found.

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

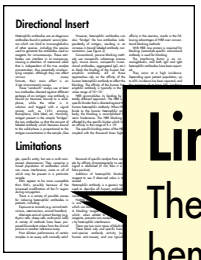
In rare cases, interference due to extremely high titers of antibodies to steptavidin and ruthenium can occur.

Elecsys Anti-HAV contains additives which minimize these effects.

IMPORTANT!

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Vaccination against hepatitis A should be considered where there is any uncertainty, and in particular if the test results borderline the cutoff (20 IU/l).

Anti-HBc Directional Insert


Directional Insert

Limitations

Limitations-interferences

The assay is unaffected by icterus (bilirubin < 25 mg/dl), hemolysis (Hb < 1.6 g/dl), lipemia (Intralipid <1,000 mg/dl) and biotin < 30 ng/ml. (criterion: correct assignment of negative and positive samples.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day) no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration 676 U/ml.

In vitro tests were performed on 19 commonly used pharmaceuticals. No interference with the assay was found.

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

In rare cases interference due to extremely high titers of antibodies to ruthenium can occur.

Elecsys Anti-HBc contains additives which minimize these effects.

Extremely high titers of antibodies to streptavidin can occur in isolated cases and cause interference.

For diagnostic purposes, the Elecsys Anti-HBc findings should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Anti-HBe Directional Insert


Directional Insert

Limitations

Limitations-interferences

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes. In rare cases, interference due to extremely high titers of antibodies to ruthenium can occur.

Troponin – I Directional Insert


Directional Insert

Limitations

Limitations of the Procedure

- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. These specimens should not be assayed with the AxSym Troponin-I assay.
- Heterophilic antibodies in human serum can react with reagent immunoglobins interfering with in vitro immunoassays. The presence of heterophilic antibodies in a patient specimen may cause anomalous values to be observed. If Troponin-I results are not consistent with other clinical observations, additional information may be required for diagnosis.

CA 15 – 3 Assay Directional Insert


Directional Insert

Limitations

Limitations of the Procedure

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse values when tested with assay kits which employ mouse monoclonal antibodies. These specimens should not be assayed with the AxSYM CA 15-3 Assay.

Toxo IgM Directional Insert


Directional Insert

Limitations

Limitations of the Procedure

3. Human anti-mouse antibodies (HAMA) may be present in samples from patients who have received immunotherapy utilizing monoclonal antibodies. Additionally, other heterophile antibodies such as human anti-goat antibodies, may be present in patient samples. This assay has been specifically formulated to minimize the effects of these antibodies on the assay. However, carefully evaluate results from patients suspected of having such antibodies.

III.
Confirming each new lot of assay reagents for blocking (release panel)

Limitations of a false positive assessment:

1. Assay dependent & Sample dependent

therefore, any given false positive sample may not be the best one to assess interference for a particular assay

2. Must be repeated regularly

"As people are given new preparations, there are going to be new antibodies with different specificities that will interfere with immunoassays. So this is a situation that never stays still, as population changes and the protein-based drugs or imaging agents they're given will change the nature of the interferences you might encounter in the sample."

– Larry Kricka, D. Phil.
Professor or Pathology University of Pennsylvania Medical Center

What the manufacturer can do proactively to reduce assay susceptibility to and consequences of false positives

- Diligently search for and obtain a large panel of false positive samples for each assay.
- Using false positive samples, develop an effective assay blocker formulation (at as low a cost as possible).
- Confirm that each new lot of blocker and each new lot of assay reagents retain effectiveness for blocking.
- Work with clinical labs to procure problematic samples in order to continuously improve the blocking formulation.
- Recognize the ineffectiveness of the product disclaimer in the package insert unless the disclaimer reaches the physician. Advise physicians directly.

What the clinical lab can do proactively to reduce the reporting and consequences of false positives

FDA

Patient → Doctor → Lab ← Manufacturer → Regulatory Agency

Clinical Use ✕ ✕ "Clinical Use" ← Clinical Trials & Approvals → Approves Product for Market

("This assay is intended to be used to test for...")

Clinical Evidence ✕ ✕ "Clinical Evidence Disclaimer" ← Product Shortcomings → Approves P.I.

"If clinical evidence does not correlate with result, consider false result."

The Lab can

- Through literature, foresee how test could be used and the false positive consequences.
- Communicate "Clinical Use Restrictions and Clinical Evidence Disclaimers" to doctors.
- Recognize that a doctor using HCG "on-label" will inevitably use it for choriocarcinoma ("off-label").

The Manufacturer can

- Through literature, foresee how test could be used and the false positive consequences; *e.g., recognize that a doctor using HCG "on-label" will inevitably use it for choriocarcinoma ("off-label").*
- Add to P.I. "Clinical Use Restrictions" (i.e. "This test not to be used for cancer diagnosis.")
- Recognize the ineffectiveness of the P.I. and communicate "Clinical Use Restrictions and Clinical Evidence Disclaimers" to doctors. (Either directly to doctors or in cooperation with labs).
- Improve the product with problematic samples and blockers.

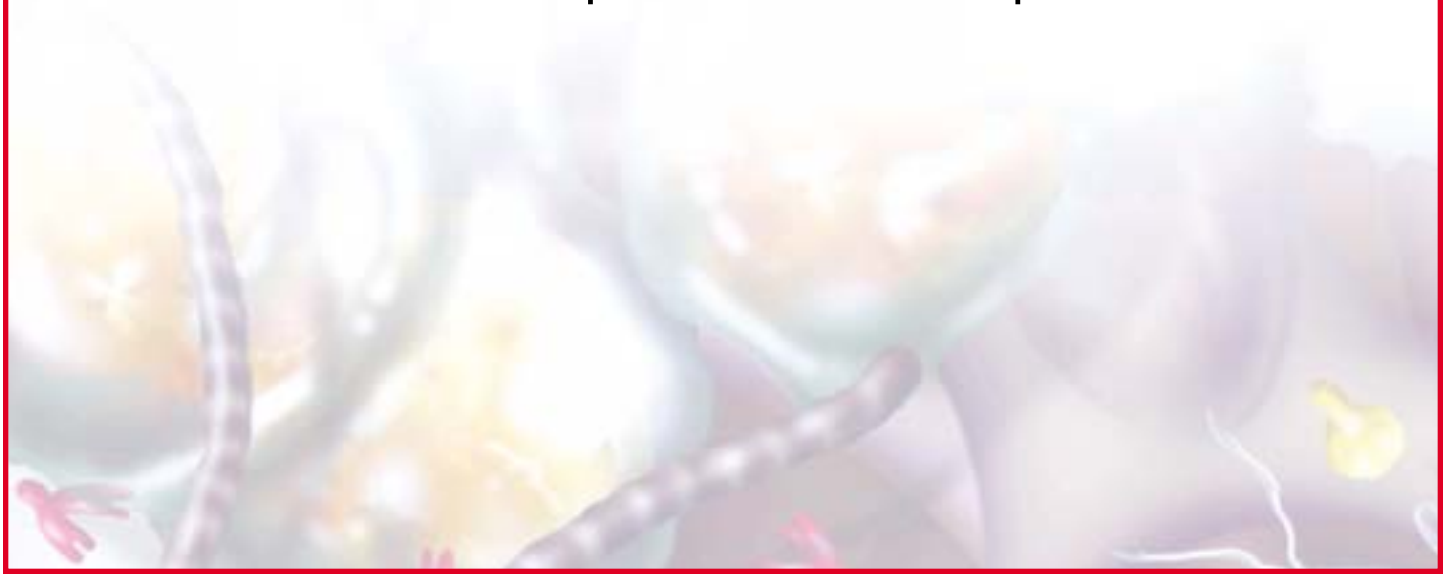
The RA can

- Through literature, foresee how test could be used and the false positive consequences.
- Require specific "Clinical NON-use claims."
- Require effective communication of P.I. "Clinical Use/Non-Use Restrictions" and "Clinical Evidence Disclaimers" to doctors.

"Most physicians never read the package insert".
(Larry Kricka)

What the clinical lab can do proactively to reduce the reporting and consequences of false positives

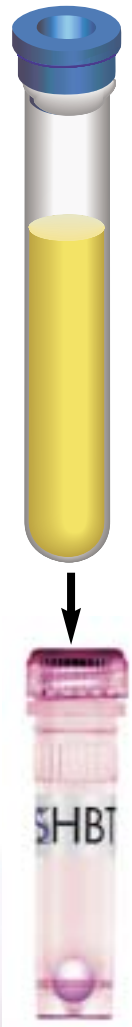
- Identify samples
  - Dilution
  - Alternate Method
  - Blocking Studies (written protocol)
- HAMA Assay
- Encourage manufacturers to use more effective blockers
- Communicate with physicians re limitations listed in package inserts
- Develop a procedures manual for handling false positives
- Document exposure and screen patients



Don't Ignore

Don't Despair

- False Positive *Samples* for Test Validation and Control
- *Reagents* for Test Improvement
- *Blocking Tubes* for Test Confirmation



For more information, contact:

SCANTIBODIES Laboratory, Inc.
9336 Abraham Way • Santee, CA 92071
Phone 1-619-258-9300 • Fax 1-619-258-9366
cs@scantibodies.com
www.scantibodies.com
Subsidiary offices in Paris and Tokyo