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Standard Operating Procedures for Serum and Plasma Collection: Early Detection Research Network Consensus Statement *Standard Operating Procedure Integration Working Group*

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Abstract

Specimen collection is an integral component of clinical research. Specimens from subjects with various stages of cancers or other conditions, as well as those without disease, are critical tools in the hunt for biomarkers, predictors, or tests that will detect serious diseases earlier or more readily than currently possible. Analytic methodologies evolve quickly. Access to high-quality specimens, collected and handled in standardized ways that minimize potential bias or confounding factors, is key to the “bench to bedside” aim of translational research. It is essential that standard operating procedures, “the how” of creating the repositories, be defined prospectively when designing clinical trials. Small differences in the processing or handling of a specimen can have dramatic effects in analytical reliability and reproducibility, especially when multiplex methods are used. A representative working group, Standard Operating Procedures Internal Working Group (SOPIWG), comprised of members from across Early Detection Research Network (EDRN) was formed to develop standard operating procedures (SOPs) for various types of specimens collected and managed for our biomarker discovery and validation work. This report presents our consensus on SOPs for the collection, processing, handling, and storage of serum and plasma for biomarker discovery and validation.

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Keywords

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Why Does It Matter How Blood Samples Are Processed?

The ultimate goal of biomarker research is to establish straightforward, cost-effective, minimally invasive clinical tests with high specificity and sensitivity for early cancer detection. Routine clinical laboratory tests for everything from potassium to prostate specific antigen (PSA) levels are highly standardized. While various institutions may use different units to define a normal range or different equipment to perform a single assay, many other parameters are strictly defined. Clinical Laboratory Improvement Amendments (CLIA) certification procedures (<http://www.fda.gov/cdrh/clia>) include detailed information about specimen collection and handling procedures that must be followed in order to achieve valid test results that can be used for diagnoses. Therefore, any potential biomarker must be able to achieve this certification for future clinical applications. Given the relative ease of collection and handling (e.g., compared to tissue) and the public perception that blood and other body fluid tests are useful, common tools for medical decision-making, the most valuable biomarkers will likely come from body fluids that are readily collected.

Standard collection methodologies are imperative if research testing results are to be translated to future clinical and diagnostic tests. The key variable in any analysis is that the case and control samples be handled in the exact same manner throughout the entire analytical process from study design and collection of samples to data analysis.^{1,2} The establishment of SOPs allows for analysis of samples collected by different groups, either because they used the same methods for specimen handling or because the specimen handling steps are documented in detail. Small differences in SOPs between groups or within groups collecting specimens could yield potentially uninterpretable or biased results due to variations at multiple steps in the collection and handling process. Variables that may impact analytic outcomes include: (1) the type of additive in the blood collection tubes; (2) sample processing times or temperatures; (3) hemolysis of the sample; (4) sample storage parameters; and (5) the number of freeze-thaw cycles.^{1,3-5} These types of differences between samples could have a significant impact on the stability of proteins or other molecules of interest in the specimens.

The Challenge Faced by a Multi-Center Consortium Devoted to Biomarker Discovery and Validation

The Early Detection Research Network (EDRN), a National Cancer Institute (NCI) initiative, was funded for systematic biomarker discovery and validation for early detection of multiple types of cancers. A consortium of investigators around the U.S. and other countries collaborate on epidemiology, sample collection and processing, biomarker discovery and validation in organ-specific cancers. The EDRN is organized into functional units responsible for the overall direction of the initiative. These units have specific tasks and scientific objectives that contribute to the overall aims and successes of the EDRN. A hallmark of the EDRN is the collaboration on all aspects of the group's mission. To further the mission of the EDRN, prospectively designed biomarker discovery and validation requires utilization of many types of human samples. Because of their frequent use in early cancer detection, establishment of standard operating procedures (SOPs) for serum and plasma were identified as the first priority of the EDRN.

The quality of human sample collection impacts analytical outcomes.^{1,3,5,6} There is significant discussion taking place in the field of biomarker discovery, particularly on the issues of sources of bias in sample collection and handling,^{1,3,7,8} but little in the way of specific instructions or SOPs that might provide points for discussion of sources of bias in study design. Here, we outline the EDRN approach to Standard Operating Procedures for collection, handling, and management of human biosamples, specifically serum and plasma.

Defining SOPs in a Multi-Institutional Consortium Environment

Standard operating procedures must provide systematic, detailed, logical instructions. They should provide enough detail to allow someone new to the procedure to understand the steps and perform the procedure consistently. Ideal SOPs are recipes, easy to follow once the right ingredients or supplies are available. They should not be teaching or “laboratory” manuals with extensive discussions and directions on theory, but rather concise step-by-step instructions.

Contributing members of the Standard Operating Procedure Internal Working Group (SOPIWG) and other EDRN members had developed their own procedures based on their individual experiences and expertise; however, there were significant variations between these methods. We acknowledged that there were numerous variables to be considered in optimum specimen management, so our first step as a group was to identify the critical variables to be considered in defining the SOPs that would be useful in our work. Important considerations were that the SOPs were generalizable, feasible and cost-effective for most groups. We addressed those variables that were thought to have the most significant impact on current proteomic platforms used by EDRN researchers. Important considerations were also the potential impact on -omic analyses and the future use of existing sample sets collected under varying protocols in combination with prospectively collected samples under the new SOPs.

General Blood Handling Considerations

The routine use and collection of blood samples for clinical diagnostics has provided general information on optimal methodologies and potential pitfalls. One such consideration is hemolysis of the specimen as it impacts the accuracy of laboratory tests, particularly chemistry tests.⁹ The release of cellular material due to hemolysis into the serum or plasma may introduce additional confounding factors in downstream analysis of such samples. Hemolysis can be prevented by careful handling techniques. We have provided instructions on optimum needle size, proper handling of the tubes, and pipetting techniques to reduce the incidence of hemolysis. Proper phlebotomy technique is also critical, but that is beyond the scope of our SOPs. We also recommend that if hemolysis (pink to red tinge in sample) is observed, this information should be recorded. It is likely that hemolyzed samples would not be used for proteomics analysis, but destroying them may be unnecessary; it is worth saving any samples, unless there are storage space constraints. Annotation of this and other pertinent information about the samples allows for a thorough discussion of potential factors that influence outcomes.

Serum Handling Considerations

Serum provides the liquid portion of the blood without cells and clotting factors and, therefore, should contain proteins and other molecules that represent the whole body system. The cells and clotting factors must be removed from the blood sample by allowing adequate time for a clot to form. Most manufacturers of collection systems for serum samples recommend 30–60 min at room temperature for a clot to form and longer if the subject was taking any kind of anticoagulant at sample collection.⁹ The selection of a collection tube was left to an individual’s discretion as long as it is without additives and designated for serum isolation by the manufacturer. This matches standard practice in clinical diagnostics. Serum samples that are allowed to sit less than 30 min are likely to retain cellular elements and other contaminants impacting future analysis. Samples that sit longer than 60 min are likely to experience lysis of

cells in the clot, releasing cellular components not usually found in serum samples.² Several investigators have tested various preanalytical sample handling parameters for proteomic serum samples.^{2,6,10} Their findings correspond well with decisions the SOPIWG made regarding SOPs for serum including time for clot formation, temperature considerations, maximum number of freeze-thaw cycles and hemolysis.

Plasma Handling Considerations

Plasma includes cellular material, providing different analytes. The dilemma facing the EDRN and any prospective biosample collection for a repository is that of unknown future analytical requirements. Plasma collection tubes contain different anticoagulants such as EDTA, heparin, or sodium citrate, and each of these additives can impact the protein makeup in the plasma and, therefore, can influence potential uses of these samples in proteomics or genomics analysis.⁵ To proceed with SOPs, the EDRN chose EDTA as the additive deemed most universally usable for our work. There was concern that heparin could interfere with some types of assays,¹¹ and was therefore not selected. Other tubes were considered including Cell Preparation Tubes (CPTs) (BD Diagnostics, Franklin Lakes, NJ) in order to isolate the buffy coat and plasma (using sodium citrate) in the same tube. We did not recommend the use of these tubes for our SOPs due to their cost and the limited need for isolating and collecting white blood cells from every subject's sample. Additional challenges with plasma include the need to properly fill the tubes during collection. The additives are calibrated to provide the optimum blood/additive ratio. Therefore, the volume of blood collected in each tube and adequate mixing of the sample into the additive are critical steps.^{5,10} The HUPO Plasma Proteome Project⁵ noted that there are too many variables to consider to make a universal statement on the best plasma SOPs for everyone to use, but the consideration of these variables in study design, along with thorough documentation of all steps of handling the samples, can minimize or even mitigate some of these pitfalls.

Temperature

Temperature is a major variable in specimen management. Everything from the temperature at collection and transport to long-term storage temperature may have major impact on the quality of the samples.^{4,5,7} As discussed above, the serum samples require ambient temperatures to form clots. Protein stability and enzyme activity are temperature-dependent as well as temperature-sensitive. The use of ice or cold packs for transport and handling steps, and quick and efficient processing of samples can minimize the degradation of proteins.^{1,4,5} The long-term storage temperature should be at least -80°C . Rai et al. suggest that liquid nitrogen storage is optimal for protein stability,⁵ but often that is not as feasible as -80°C . Several investigators^{1,4,5} have shown that the number of freeze-thaw cycles a specimen undergoes has a dramatic negative effect on the quality of the specimen. Serum and plasma specimens are of better quality for analysis if smaller volume aliquots are initially prepared rather than larger ones that have to be thawed, handled, and refrozen, perhaps multiple times.^{1,4} Indeed, the ability to provide ready-made aliquots without additional handling steps facilitates the sharing of samples and provides multiple replicates that were handled in an identical manner.

Potential temperature issues arise in the transportation of samples within and between facilities. Care must be taken to ensure that the serum and plasma samples have sufficient dry ice for the expected duration of the trip, whether it is across the hall or across the country. Samples that have thawed (e.g., due to rerouted flights or left out too long during relabeling) should not be used, so steps must be taken to protect the specimens from delays that can and do happen. To address this potential pitfall, EDRN SOPs require the addition of sufficient dry ice to keep samples frozen for an additional 24 h beyond the expected shipment duration and discuss the need to have backup plans for freezers.

Light-Sensitivity

Some analytes can be broken down by exposure to natural or fluorescent light.¹⁰ Examples in clinical chemistry include bilirubin and beta-carotene,¹⁰ but there are many other compounds that are potentially adversely affected by light exposure. Blood tubes wrapped in aluminum foil during transport and handling, and the use of opaque boxes for storage can minimize the potential damage to photosensitive analytes.

Reaching Consensus

To begin this process, EDRN collaborative groups focusing on different organ systems (Breast & Gynecological Cancers, Colorectal and Other Gastrointestinal Cancers, Lung and Upper Aerodigestive Cancers, and Prostate and Other Urologic Cancers) provided SOPs for their blood samples. One set of SOPs was selected as the starting point. Conference calls were held monthly to discuss decisions to be made on the first set of SOPs for serum and plasma. Similarities and differences between the serum/plasma SOPs were charted. While every step was reviewed and discussed, those variables deemed to be critical generated more significant debate.

Major SOP differences can be summarized as times, temperatures, and types of additives. Times varied for clot formation (range 30 min to 5 h), time allowed at various temperatures until aliquoting and freezing, and time for centrifuging. Temperatures varied (in conjunction with times) from room temperature to 4 to -80°C at stages from collection, clotting, holding, centrifuging, and long-term storage. Finally, all 3 types of common anticlotting additives (EDTA, heparin and sodium citrate) were used for plasma collection. We consulted with end-users and commercial sources (such as BD.com) to help develop consensus on the critical issues. Specifically, we sought the input of various EDRN units such as Biomarker Reference Laboratories (BRLs), members of each organ site collaborative group, and proteomic and genomic experts in the EDRN. Several drafts were reviewed until consensus through compromise was achieved. A final SOP for plasma processing and a final SOP for serum processing were circulated for approval by all EDRN members. The SOPs are posted on the EDRN Web site (<http://edrn.nci.nih.gov/resources/standard-operating-procedures/biological-specimens>). Additional discussions reviewed the implementation process for each group based on their existing and planned studies.

One difference between serum methods was in the processing time and temperature of the blood specimens. As noted above, in order to obtain serum of high quality, blood samples should be allowed time to form a clot at room temperature for 30–60 min.¹⁰ The contributed SOPs had a range of time to allow clot formation that was reasonably consistent at 30 min minimum to 60 min maximum (with one at up to 5 h), and the final SOP directs users to allow 30–60 min to clot, then process in a centrifuge or hold at 4°C for *no more* than 4 h. The contributed SOPs had various times allowed for holding at 4°C until processing (range 0–26 h). The consensus SOP reflects a compromise between the most optimal specimen processing (processing immediately after clot formation) and the reality of collecting and processing samples in clinical settings with busy staff. In cases where samples can be processed immediately, the SOPs allow for that, while setting a maximum acceptable time limit. Data regarding the time and temperature during processing need to be recorded, as well as specific handling steps. These data are critical for the complete analysis of the collected samples.

The most significant differences among the different collaborative groups were noted for plasma collection. Contributing groups used different types of blood collection tubes, different holding temperatures (e.g., room temperature or 4°C) and different time periods prior to centrifugation (from 2 h up to 26 h after collection). The wide array of differences in processing

the samples limits the generalizability of assays within and between organ groups. Our final SOPs settled on EDTA tubes with immediate processing or holding no more than 4 h at 4 °C prior to processing. The decisions on time and temperature variables for plasma were much easier after the serum processing parameters were agreed upon. The decision on which anticoagulant to use was more difficult. Each anticoagulant has advantages and disadvantages depending on the intended uses for the plasma, and there is no universal agreement on the tube of choice. EDTA was selected as it was determined to be the least likely to interfere with the majority of assays in use currently.

Additional variables, albeit seemingly minor details, can impact the success of an assay. The use of glass versus plastic blood collection tubes is one such variable.¹² In the interest of personnel safety, suppliers provide plastic blood collection tubes for routine clinical use instead of glass tubes;¹⁰ however, it is not known if there is a potential for leaching of plastic ingredients into the specimen during collection that could impact sensitive assays. Stankovic and Parmar¹² discuss the potential negative impact on clinical assays due to additional materials such as silica particles or polyvinylpyrrolidone to plastic collection tubes. Clinical laboratories and the corresponding common laboratory tests have established protocols, quality assurance steps, and significant testing by suppliers to minimize the damage from a major change such as from glass to plastic.^{10,12} Existing samples were most likely to have been collected in glass blood collection tubes; switching to plastic could introduce potential bias. Our SOPs recommend the use of glass tubes due to the potential problems of leaching from plastic or switching from glass.

While the EDRN strongly encourages the use of these SOPs for developing repositories of member groups, it was understood that there would be reasons why this would not be feasible for every group on every study. The implementation process acknowledged this constraint, and groups agreed to implement the necessary changes to their own SOPs as they could. Studies ongoing under particular specimen SOPs did not change methods midstream, as that would clearly cause significant detrimental differences between samples collected on the same protocol. Groups that are implementing new studies are using the EDRN SOPs so that samples collected from different groups going forward can be assayed together. The process of developing these SOPs also encouraged continued communication between those groups collecting samples and those groups using samples within the EDRN. This discussion served to re-emphasize the need for detailed documentation of the specimen handling steps from collection to assay and strict adherence to the sample processing protocols in use. Attention to these two critical details should yield quality, well-annotated samples for biomarker discovery and validation work.

Challenges, Limitations, and Weaknesses

Institutional Review Boards and Human Subject Research Protections

Since samples come from research volunteers, properly obtained informed consent prior to sample collection is imperative. Even before the new HIPAA privacy rules took effect April 14, 2003, Institutional Review Boards (IRBs) and investigators were struggling with how to obtain truly “informed” consent. Questions, such as what to tell subjects about the use, labeling, and long-term disposition of their samples, who “owns” the samples (institutions, investigators, sponsors) or are there limitations on how the samples can be used, needed to be addressed in the consent document and consent discussions. Hindsight (and lawsuits) prompted many institutions to develop policies and guidance documents for sample collection and sample management to address these questions; in some situations, this has added more barriers to the sample collection process. Privacy Boards (responsible for HIPAA compliance) and IRBs are focused on potential HIPAA issues in clinical research design, especially in terms of specimens and data collected. Interestingly, most sample collection protocols and procedures are

considered to be of minimal risk to subjects, but the questions and their answers make for some of the more challenging IRB decisions. In any case, thoughtful attention to these kinds of questions during study design and initial application is critical.

Health Insurance Portability and Accountability Act (HIPAA)

After April 13, 2003, the enforcement of HIPAA had a major impact on clinical research. Stricter restrictions on the use and disclosure of personal health information or protected health information (PHI) changed (and significantly limited) the way research data could be gathered and used. Researchers must tell subjects how their “information” will be used—everything from what kind of information would be on the research specimen label to how the sample would or would not be used. Most institutions combine this information with the consent form as IRBs often serve the dual function of IRB and Privacy Board. Suffice it to say HIPAA considerations have had a major impact (both positive and negative) on sample and data collection and management. Potential HIPAA violations lurk in research laboratories and “simple” blood draw studies. Gone are the days when a name, a full date of birth, or a medical record number could be used on sample and data forms. Many investigators have had to redesign their specimen management systems, from vial labeling to freezer management to store samples without PHI in compliance with HIPAA. These issues are important to address from the beginning of a new study design. HIPAA regulations impact everything from annotation of sample details to tracking multiple aliquots from a single subject. When the samples must be labeled with a code and the subject’s PHI link is elsewhere, the potential for mislabeling, mixing up, or misplacing samples markedly increases.

Feasibility

Upon the basis of optimal specimen handling conditions and regulatory issues, the group considered other questions in the development of the SOPs. What are the common types of laboratory equipment to which a majority of investigators would have access (e.g., -80°C freezers or -20°C freezers)? Are we introducing any significant barriers to following the SOPs by the types of equipment or conditions we are defining? Are the SOP expectations reasonable given the assumption of some limited resources (personnel, money, time)? Is this truly feasible in a clinical setting? The SOPs developed and approved by the EDRN are currently being disseminated. During the review process, we found that this approach is feasible in the EDRN centers. We would expect these SOPs would be feasible in a majority of clinical research settings.

Future Uses of Today’s Samples

The field of proteomics is rapidly evolving with new technologies and new standards. Often samples are collected and stored for a long period of time (sometimes years) before being used. Sample handling methodologies must be defined before the technology to assay them exists. Therefore, the well-annotated, “perfectly” collected samples of today, may not be suitable for the technology of tomorrow. Although this particular scenario has not yet had a dramatic impact on the existing sample repositories, it is a sobering consideration, given that virtually every major clinical research initiative from EDRN to cooperative groups are banking serum, plasma, tissue, and urine for biomarker research. The decisions being made now on sample collection methodologies and storage parameters will have a significant impact on the quality and utility of materials available for the technology of tomorrow. Recent publications highlight the need for consideration of at least a couple of basic points for handling specimens for proteomics work in order to provide useful samples.^{1,2,5} These are consideration of the type of specimen needed and the proper tube to collect the specimen, how to consistently handle and process the samples and a thorough documentation of the steps a particular sample experienced from collection to assay. Issues of uniform collection, handling, and storing of samples do not just

lead to hypothetical problems in studies. For example, differences in sample collection and handling between cases and controls lead to bias in a study of the early detection of prostate cancer. This bias was identified in an EDRN validation study which could not replicate previous results.³

Bias

There are many ways to introduce bias at steps from study design to analysis.^{1,3,8} It is virtually impossible to design a specimen collection SOP that addresses every possible source of bias prospectively. One major example of possible bias is that the methods chosen for sample handling could have a bias inherent in the design rendering all samples equally useless for a specific study. Another example, as McLerran et al.³ openly acknowledge, is the age of the samples being assayed. While SOPs cannot overcome this barrier alone, SOPs can certainly reduce some of the risk of bias in sample handling. Combining a systematic approach to specimen handling, education about the issues herein, thoughtful discussion and debate (including publications), and annotation of a sample's history allows acknowledgment of the potential sources of bias.

Expected Outcomes from Using SOPs

Any validated biomarker for cancer detection should include specifications for its use in a clinical setting including preanalytical variables in collection, processing and storage in order to yield a relevant test result for a patient. The EDRN consensus SOPs address consistent blood specimen handling and processing steps that provide a basis on which to build a clinically valid test for early detection of cancers. It is critical to record sample handling and processing information so that samples have associated processing data available; these data can be used in analysis to cull out potential confounding factors and provide alternate explanations for experimental results. Samples collected under the EDRN SOPs with careful documentation of collection and processing variables within the recommended SOP will help answer important questions, including the impact of differences in specimen handling within and between samples from cancers, normals, and other precancerous conditions.

Summary

Often specimen collection is perceived as the “easy part” of a protocol, yet validation of biomarkers relies on the control of bias introduced at any step in the collection process. Cases and controls must have matching populations, collection, processing, storage, and shipping procedures. This is necessary in order to ensure that any differences identified between the groups of samples are due to real differences between the disease state and controls and not from bias in handling of samples. The EDRN working group SOP project has increased awareness of issues in sample collection and the potential downstream impact (positive and negative) on biomarker discovery, analysis, and validation work. We identified a need for SOPs for other types of samples as well as identifying a wide variety of functional and technical issues related to biomarker research validation and clinical testing. This process empowered the clinical epidemiology stakeholders (from study coordinators and processing laboratories to biomarker discovery laboratories) with an appreciation of the importance of their role in specimen management.

In this discussion, we have not addressed “population” differences, an important source of sample bias which should be controlled through protocol design. Protocol designs must deal with racial differences, stress, fasting-fed states, and comorbid conditions to the disease of interest. These issues are not part of sample collection SOPs and are not considered within the scope of the EDRN SOP project.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Grizzle WE, Semmes OJ, Bigbee W, Zhu L, Malik G, Oelschlager DK, Manne B, Manne U. *Cancer Inf* 2005;1(1):86–97.
2. Timms JF, Arslan-Low E, Gentry-Maharaj A, Luo Z, T'Jampens D, Podust VN, Ford J, Fung ET, Gammerman A, Jacobs I, Menon U. *Clin Chem* 2007;53(4):645–56. [PubMed: 17303688]
3. McLerran D, Grizzle WE, Feng Z, Bigbee WL, Banez LL, Cazares LH, Chan DW, Diaz J, Izbicka E, Kagan J, Malehorn DE, Malik G, Oelschlager D, Partin A, Randolph T, Rosenzweig N, Srivastava S, Srivastava S, Thompson IM, Thornquist M, Troyer D, Yasui Y, Zhang Z, Zhu L, Semmes OJ. *Clin Chem* 2008;54(1):44–52. [PubMed: 17981926]
4. Mitchell BL, Yasui Y, Li CI, Fitzpatrick AL, Lampe PD. *Cancer Inf* 2005;1(1):98–104.
5. Rai AJ, Gelfand CA, Haywood BC, Warunek DJ, Yi J, Schuchard MD, Mehig RJ, Cockrill SL, Scott GB, Tammen H, Schulz-Knappe P, Speicher DW, Vitzthum F, Haab BB, Siest G, Chan DW. *Proteomics* 2005;5(13):3262–77. [PubMed: 16052621]
6. Hsieh SY, Chen RK, Pan YH, Lee HL. *Proteomics* 2006;6(10):3189–98. [PubMed: 16586434]
7. Mischak H, Apweiler R, Banks RE, Conaway M, Coon J, Dominiczak A, Ehrlich JHH, Fliser D, Girolami M, Hermjakob H, Hochstrasser D, Jankowski J, Julian BA, Kolch W, Massy ZA, Neusuess C, Novak J, Peter K, Rossing K, Schanstra J, Semmes OJ, Theodorescu D, Thongboonkerd V, Weissinger EM, Van Eyk JE, Yamamoto T. *Proteomics Clin Appl* 2007;1(2):148–156.
8. Ransohoff DF. *J Clin Epidemiol* 2007;60(12):1205–19. [PubMed: 17998073]
9. Arzoumanian L. *BD Technical Services News Bulletin* 2003;2(2)
10. Magee LS. *Lab Notes* 2005;15(1):1–4.
11. Yokota M, Tatsumi N, Nathalang O, Yamada T, Tsuda I. *J Clin Lab Anal* 1999;13(3):133–40. [PubMed: 10323479]
12. Stankovic AK, Parmar G. *Clin Chem* 2006;52(8):1627–8. [PubMed: 16873316]