Evaluation of biological specimen acceptability in a complex clinical laboratory before and after implementing automated grading serum indices

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ABSTRACT

Continuous monitoring of specimen acceptability, collection and transport can result in the prompt identification and correction of problems, leading to improved patient care and a reduction in unnecessary redraws and delays in reporting results. This study aims to identify unacceptable blood specimens and to calculate the specimen rejection rate (SRR) before and after the implementation of automated checks of serum indices. This study was conducted between January 2009 and December 2010. The number of rejected specimens, location and reason for rejection were recorded. The Architect c8000 analyser (Abbott, Illinois, USA) was used to assess serum indices based on characteristic spectral patterns and mathematical manipulations of absorbance values measured at several wavelengths. The SRR was calculated, and the target cut-off value for the SRR was <0.5%, as established by the College of American Pathologists (CAP). The SRR values were 0.13% and 0.21% for the years 2009 and 2010, respectively. Haemolysis was the most significant reason for sample rejection, with cumulative rejection rates (CRR) of 49.3% and 61.4% for 2009 and 2010, respectively. Adult intensive care units (ICUs) had the most sample rejections (23.5%), followed by neonatal ICUs (13.8%), cardiac ICUs (13%), paediatric ICUs (10.8%) and long-term wards (10.5%), of which 60%, 79%, 84.9%, 36.6 % and 75%, respectively, of the rejected samples were haemolysed. The increase in rejected samples may be due to an improvement in staff awareness of sample rejection, aided by automatic sample integrity grading by automated chemistry analysis systems.

KEY WORDS: Hemolysis.
Pre-analysis.
Serum indices.
Specimen rejection.

Introduction

Most laboratory errors are due to preanalytical factors (46–68.2% of total errors), whereas a high error rate (18.5–47% of total errors) has also been found in the post-analytical phase. In contrast, lower error rates have been found in the analytical phase (7–13%). Therefore, clinical laboratories must improve the situation in the preanalytical phase, which is highly susceptible to errors.[1,2] The three major endogenous compounds that often interfere with laboratory results are haemoglobin, bilirubin and lipids, the so-called serum indices.[3–5]

The frequency of interference in clinical laboratory analyses is very difficult to determine.[3] In principle, interference can be reduced by the use of adequately blanked analytical methods.[6] Many chemistry analysers have the capability to detect haemolysis, icterus and lipaemia in samples and to produce semiquantitative unit less index values for haemoglobin, bilirubin or triglycerides, but these serum index functions are not intended for diagnostic purposes. With increasing amounts of interfering compounds, increasing index values are generated, which are linearly correlated with the amount of interference present.

Haemolysis occurs as a result of the breakdown of red blood cell (RBC) membranes, which causes the release of haemoglobin and other internal components into the surrounding fluid. Haemolysis can be detected visually as a pink/red tinge in serum or plasma.[7] Haemolysis is a common occurrence in serum samples and may compromise test parameters. It can originate from *in vivo* haemolysis, which may be due to pathological conditions, such as autoimmune haemolytic anaemia or transfusion reaction, or *in vitro* haemolysis, which may be due to improper specimen collection, specimen processing or specimen transport.[7]

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In some reports, haemolysis, the most common reason for rejection, accounts for 60% of rejected specimens, a figure five-fold higher than the second most common cause,[8,9] and is attributable to *in vitro* processes involving incorrect sampling procedures or transport. Cellular contents can falsely increase values for some plasma constituents, such as potassium, lactate dehydrogenase and aspartate aminotransferase.[10] Moreover, haemolysis produces spectrophotometric interference with other laboratory methods.

Simundic *et al*. reported that preanalytical errors were the most common within the total testing process, and

haemolysis was recognised as one of the most prevalent preanalytical errors in clinical laboratory testing. They indicated that visual detection of haemolysis is arbitrary and therefore mostly unreliable as it may over-estimate and under-estimate the actual prevalence of haemolysed serum specimens (i.e., trained observers are unable to rank accurately the degree of interference in serum). Elevated bilirubin may further impair the ability to detect haemolysis by visual inspection and therefore lead to serious under-estimation of haemolysis in neonatal samples where elevated bilirubin concentration is common.

Recent advances in laboratory technology have led to an increasing trend in the automation of various preanalytical processes into large preanalytical modules. Such automated laboratory systems offer the automated detection of serum indices. This is advantageous due to the increased reproducibility and the improvement in detection of mildly haemolysed specimens (serum haemoglobin <0.6 g/L). These platforms commonly use semiquantitative spectrophotometric measurement and grade interfering substances into several categories. However, various analytical platforms may have different decision thresholds for serum indices. Moreover, systems may differ in their assay parameters and the degree of the interference of the specific interfering substance. Therefore, further efforts should focus on standardising the mean of reporting the haemolysis index, especially when this important parameter is used to obtain meaningful information on the quality of sample collection.[11]

The occurrence of *in vitro* haemolysis mainly depends on the way in which blood samples are drawn and treated; it may arise from the blood being forced through a fine needle[12] or through the needle of a syringe into a tube. It may also be caused by the tube being shaken too vigorously and/or the centrifugation of blood specimens before clotting is complete. However, *in vivo* haemolysis may have many causes.[12]

In 2009, interference was detected by visual inspection, and the processing of the finding (e.g., sample rejection, correction, or addition of a comment to the report) was determined by the technician handling the specimen. If the correct action was taken, it was recorded on the laboratory information system (LIS). Automated spectrophotometric detection of interference by the chemistry analyser and decision rules for the systematic, although still manual, handling of specimens based on tolerance tables for clinically relevant interference was introduced in the first half of 2010. The algorithm for automated decision-making by the LIS for the processing of serum interference was introduced, and all reported actions were automatically archived in the LIS.[13]

A high degree of variability in the training, skill and frequency of phlebotomy practice of non-laboratory staff is a major factor contributing to haemolysis rates in many facilities. Haemolysis is the major cause of specimen rejection, as shown by the College of American Pathologists (CAP) Chemistry Specimen Acceptance Q-Probes study.[8] Some facilities have reverted to the use of centralised phlebotomy teams to alleviate the quality issues associated with poor collection. Haemolysis leads to a higher rate of rejected specimens and is a cause of frustration in laboratories and in-patient units. Often, rejected samples and inaccurate results are attributed to "laboratory errors", with the blame usually placed on the medical technologist. Rarely is a connection made between improperly collected specimens and inaccurate laboratory results.

This study evaluates the causes of haemolysis in samples received in the chemistry section of the Department of Laboratory Medicine, King Abdulaziz Medical Center in Riyadh, Saudi Arabia. In addition, it assesses the effect of interference by haemolysis, icterus or lipaemia on various analytes using interference data provided by the analyser manufacturer.

To determine whether the unacceptable result rate attributable to endogenous interference improves over time, a retrospective analysis is performed to study the effect of the stepwise introduction of systematic detection and the subsequent automatic rule-based algorithm for actions to be taken on behalf of the clinician (correction of the result by adding a comment to the report) or the patient (collection of a new sample).

Table 1. Reasons for specimen rejection and the cumulative rejection rate (CRR) for 2009 and 2010.

Reason for specimen rejection	CRR 2009	CRR 2010
Haemolysed	49.3%	61.3%
Contaminated	23%	20.2%
Quantity not sufficient (QNS)	13.7%	12.0%
Lost	3.2%	0.8%
Unacceptable variance (delta check)	3.0%	1.5%
Wrong collection container	0.6%	0.2%
Clotted	0.5%	0.5%
Improper storage temperature	0.2%	0.0
Unlabelled	0.3%	0.0
Mislabelled	0.1%	0.2%
Other	6.1%	3.3%

Materials and methods

This study was conducted over a two-year period (January 2009 to December 2010) at the King Abdulaziz Medical Center, which is affiliated to the King Saud Bin Abdulaziz University for Health Sciences in Riyadh, Saudi Arabia.

The number of rejected specimens, the location and the reasons for rejection were recorded. Reasons for sample rejection included haemolysis, contamination, quantity not sufficient (QNS), lost sample, unacceptable variance (delta check; which requires a comparison of current and previous results), the use of the wrong collection container, clotted samples, improper storage temperature, unlabelled sample and mislabelled sample.

During 2009, inspection of serum indices was performed manually by laboratory staff. However, in 2010 the Architect c8000 chemistry automated analyser (Abbott, USA) was used to assess levels of serum indices (i.e, haemoglobin, bilirubin and triglyceride), based on a characteristic spectral pattern and mathematical manipulation of absorbance values measured at several wavelengths. The evaluation between inspection of serum indices manually and after employing

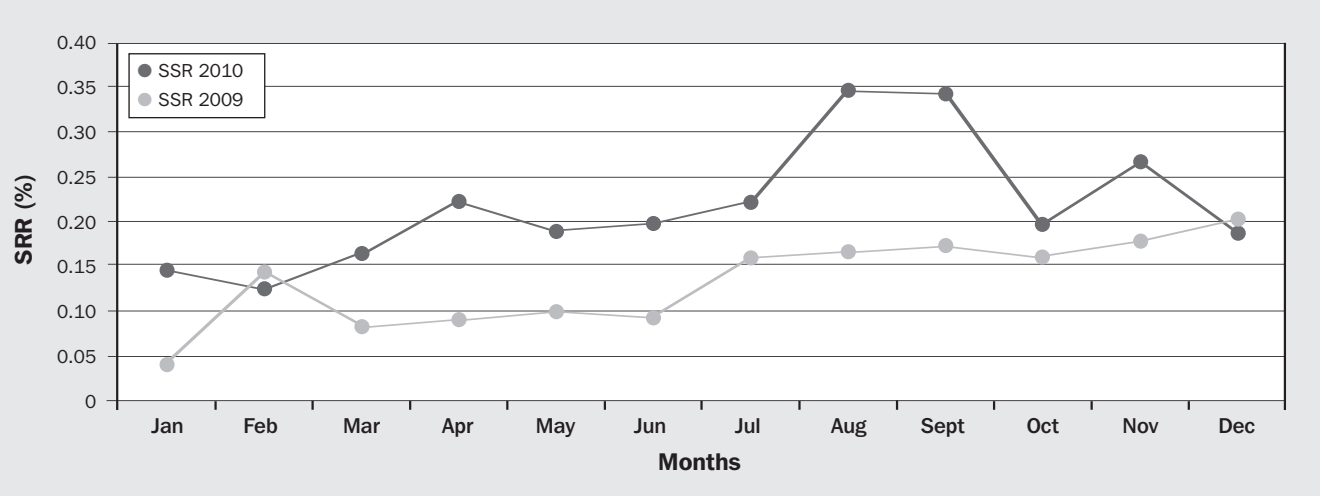

Fig. 1. Monthly specimen rejection rate (SRR) for 2009 and 2010.

the automated grading system was compared by assessing the specimen rejection rate (SRR). The SRR was calculated as the percentage of rejected samples per total samples received. The target cut-off value for the SRR was <0.5%, as established by the College of American Pathologists (CAP).8

The process of identifying and classifying the requesting locations of haemolysed samples was carried out for a period of seven months (June to December 2010). A total of 760 rejected samples were monitored, of which 466 were haemolysed (61.3%) and 294 (38.7%) were rejected due to other reasons.

The specimens studied, collected by clinicians or nurses from hospitalised patients, were received from internal medicine (28%) and surgery (21%), intensive care units (ICUs; 23%), emergency departments (16%) and organ transplantation (9%). According to the study protocol, each time haemolysis was identified visually, even if only slight, the laboratory contacted the phlebotomists to determine the procedure and technique used to draw blood, and to obtain information on the transportation, preservation and storage of the specimens. If no errors were identified in these procedures and *in vivo* haemolysis was not suspected clinically, serum haptoglobin levels were measured to confirm the presence of acute haemolysis, which was evaluated clinically and then confirmed by further analysis.

Table 2. Site and location of rejected haemolysed samples.

Location	Total rejected samples
Adult ICU	23.5%
NICU	13.8%
Cardiac ICU	13.0%
Paediatric ICU	10.8%
Long-term ward	10.5%
Paediatric ward	7.6%
A&E	4.0%
Medical ward	4.0%
Labour and delivery	3.8%
Out-patient clinic	2.24%
Other location	3.6%

Results

Figure 1 shows the percentage SRR on a monthly basis for 2009 and 2010. The overall rate was found to be 0.13% and 0.21% for 2009 and 2010, respectively (61.5% increase). Figure 2 and Table 1 show the reasons and the cumulative rejection rate (CRR) for the specimens rejected. The most common reasons were haemolysis, with rates of 49.3% and 61.3% during 2009 and 2010, respectively, followed by sample contamination (23% and 20.2%, respectively) and quantity not sufficient (QNS; 13.7% and 12.0%, respectively).

There was an increase of 100% and 24.3% in the number of mislabelled and haemolysed samples, respectively, during 2010. However, decreases in QNS, contamination, lost sample, delta check, use of the wrong collection container, improper sample temperature, unlabelled sample and other rejection causes were 12.4%, 12%, 75%, 50%, 66%, 100%, 100% and 46%, respectively.

Haemolysis was the most significant reason for rejection, with CRR of 49.3% and 61.4% for 2009 and 2010, respectively. Table 2 shows the collection sites for the rejected samples.

Discussion

Jones *et al*. determined the rejection frequency and reasons for rejection of chemistry specimens using the College of American Pathologists Q-Probes programs.[8] They collected prospective data regarding rejected chemistry specimens in 453 laboratories, and found that of the 10,709,701 chemistry specimens submitted to the participating laboratories during the data collection period, 37,208 (0.35%) were rejected prior to testing. The most frequent reason for rejection was haemolysis, which occurred five times more frequently than the second most cited reason, which was insufficient specimen quantity to perform the test. On further examination, it was found that a higher percentage of rejected specimens was collected in microcollection tubes than in other containers. Compared to frequency with which they collect specimens, laboratory personnel submitted significantly fewer rejected specimens than other in-hospital personnel groups, and slightly more than out-of-hospital, non-laboratory personnel. Poorest performance was demonstrated by other in-hospital, non-laboratory

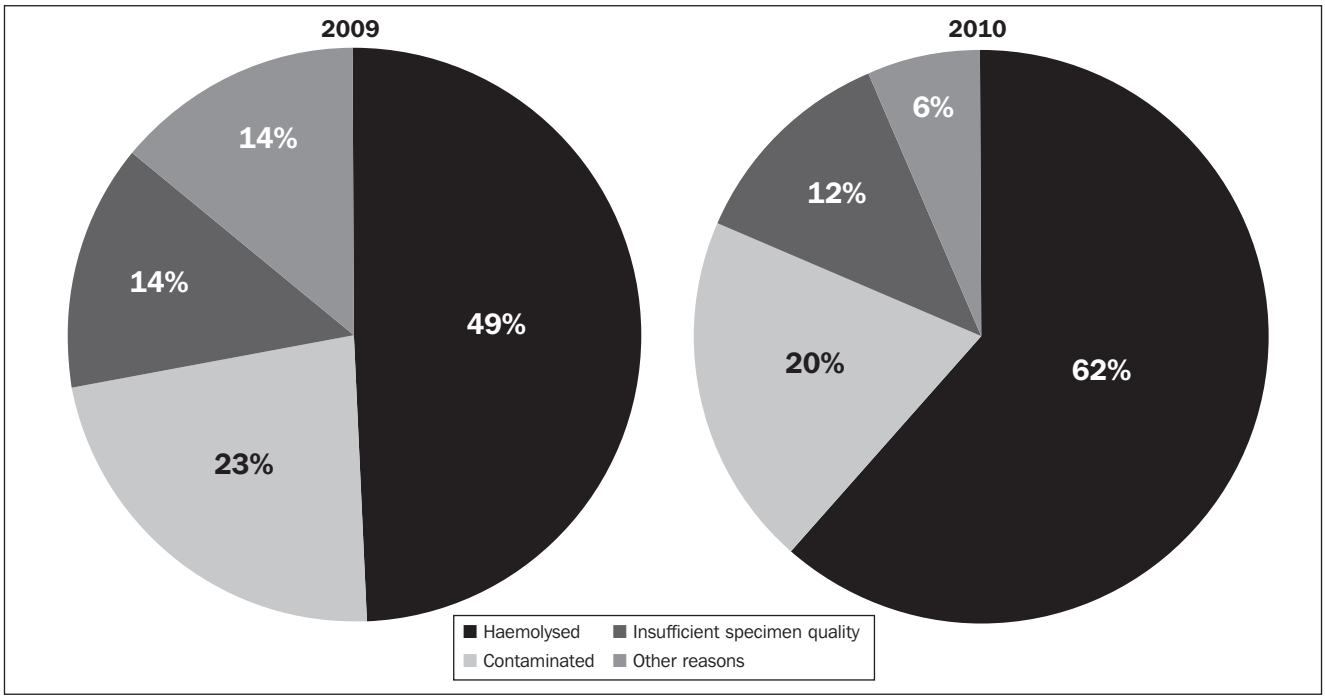

Fig. 2. The major reasons for specimen rejection, 2009 and 2010.

personnel. Serum and plasma oxalate/fluoride specimens showed significantly lower rejection rates compared to other specimen types. The relative rejection rates were higher for non-gel tubes and lower for syringes compared to gel tubes.[8]

The introduction of automated spectrophotometric detection of serum indices increased the detection rate of relevant haemolysis by 24.3% in the present study. In the study by Henricus *et al*., a six-fold increase in haemolysis was observed when they compared manual inspection to an automated processing of serum indices.[14] However, the automated detection performed and the quality improvement observed depends on the validity of the automated procedure and can be used as a blueprint for processing test results in general. Every test result in the authors' hospital is now evaluated automatically in real time against predetermined tolerance limits for the extent of interfering substances, and the algorithm is designed to be almost independent of technician input. This is in full agreement with the studies of Simundic *et al*.[14] and Carraro *et al*.[2]

Simundic *et al*. assessed the comparability of automated spectrophotometric detection and visual inspection of lipaemic, icteric and haemolysed samples. They reported that visual inspection is highly unreliable and should be replaced by automated systems that report serum indices. Visual inspection is time-consuming, highly subjective and not standardised.[11]

Lippi *et al*. indicated that haemolysed specimens are a frequent occurrence in clinical laboratories and that their prevalence can be as high as 3.3% of all routine samples, accounting for 40–70% of all unsuitable specimens identified, which is nearly five times higher than those of other causes (e.g., insufficient, incorrect and clotted samples).[15]

In a retrospective cross-sectional study, Stark *et al*. observed that clinical laboratories that participate in the CAP Q-track programs to track laboratory quality reported aggregated specimen rejection rates of 0.30–0.83%.[16] They calculated the proportion of rejected specimens stratified by the point of collection and found that the proportion of rejected specimens collected from emergency department and in-patient services were two-fold and five-fold higher, respectively, as compared to out-patient services.

In the five-year retrospective analysis conducted by Alsina *et al*.[17] it was determined that 81% of rejections arose as a result of specimen not received (37.5%), haemolysis (29.3%) and clotted sample (14.4%). Dale and Novis prospectively evaluated the successful encounters and percentage of unsuitable specimens collected from clinical laboratories participating in the CAP Q-Probes laboratory improvement program and characterised the outcome of out-patient phlebotomies for three months or until 20 unsuccessful phlebotomy encounters occurred.[18] Using a questionnaire, participants provided information about test ordering, patient preparation and specimen collection. They found that only 2153 of the specimens (0.3%) were unsuitable. These samples were haemolysed (18.1%), of insufficient quantity (16.0%), clotted (13.4%), lost or not received by the laboratory (11.5%), inadequately labelled (5.8%), at variance with previous or expected results (4.8%), or unacceptable for other reasons (31.1%). However, in a recent study,[19] Guimaraes *et al*. reported that clot was found to be the major cause of rejection of samples (43.8%), followed by insufficient sample volume (24%) and haemolysed sample (17.9%).

Söderberg *et al*.[20] found that samples from primary health centres (PHCs) were haemolysed over six times (95% confidence interval [CI: 4.0–9.2]) more often compared with other centres. A notable difference in haemolysed samples was found between the emergency department staffed by emergency medicine clinicians and those staffed by primary healthcare clinicians (34.8% *vs*. 11.3%, $P<0.001$).[20] Francis *et al*.[21] observed a decrease in mislabelling errors when they

applied radiofrequency identification (RFID) technology to specimen bottles.

Sodi *et al*.[22] reported that the pneumatic tube system (PTS) has been implicated in inducing haemolysis. Their results suggest that plain serum samples are more susceptible to haemolysis than are other sample types when transported via PTS ($P<0.0001$). When comparing serum with gel samples, plain serum samples are more prone to haemolysis ($P<0.001$). This suggests that the gel may confer some protection against haemolysis. They recommended that each hospital assess its PTS for blood haemolysis. However, different hospitals vary with respect to system configurations and use different sample types. In a future study, the present authors plan to investigate the PTS to assess whether or not haemolysis is a recurring problem in any of the sample types transported.

Lowe *et al*. found that haemolysis rate differs depending on the blood collection technique used and is lower for venepuncture compared to intravenous catheterisation in emergency departments.[23] In another study, by Bush *et al*., it was found that haemolysis occurs more frequently in line draws compared to venepuncture samples.[24] □

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References

1 Plebani M, Carraro P. Mistakes in a stat laboratory: types and frequency. *Clin Chem* 1997; **43** (8 Pt 1): 1348–51.
2 Carraro P, Servidio G, Plebani M. Hemolyzed specimens: a reason for rejection or a clinical challenge? *Clin Chem* 2000; **46** (2): 306–7.
3 Kroll MH, Elin RJ. Interference with clinical laboratory analyses. *Clin Chem* 1994; **40** (11 Pt 1): 1996–2005. Erratum in *Clin Chem* 1995; **41** (5): 770.
4 Behrendt H. *Chemistry of erythrocytes*. Springfield, IL: Charles C Thomas, 1957.
5 Caraway WT. Chemical and diagnostic specificity of laboratory tests. Effects of hemolysis, lipemia, anticoagulants, medications, contaminants, and other variables. *Am J Clin Pathol* 1962; **37**: 445–64.
6 Young DS. *Effect of drugs on clinical laboratory tests*. Washington, DC: AACC, 1990.
7 Lemery L. Oh, No! It's hemolyzed! What, Why, Who, How? *Advance for Medical Laboratory Professionals* 1998; Feb 15: 24–5 (http://laboratorian.advanceweb.com/Article/Oh-No-Its-Hemolyzed.aspx).
8 Jones BA, Calam RR, Howanitz PJ. Chemistry specimen acceptability: a College of American Pathologists Q-Probes study of 453 laboratories. *Arch Pathol Lab Med* 1997; **121** (1): 19–26.
9 Jay D, Provasek D. Characterization and mathematical correction of hemolysis interference in selected Hitachi 717 assays. *Clin Chem* 1993; **39** (9): 1804–10.
10 Pai SH, Cyr-Manthey M. Effects of hemolysis on chemistry tests. *Lab Med* 1991; **22**: 408–10.
11 Simundic A-M, Topic E, Nikolac N, Lippi G. Hemolysis detection and management of hemolyzed specimens. *Biochemia Medica* 2010; **20** (2): 154–9.
12 Kroebke J, McFarland E, Mein M, Winkler B, Slockbower JM. Venepuncture procedures. In: Slockbower JM, Blumenfeld TA eds. *Collection and handling of laboratory specimens*. Philadelphia: JB Lippincott, 1983: 32–4.
13 Vermeer HJ, Thomassen E, de Jonge N. Automated processing of serum indices used for interference detection by the laboratory information system. *Clin Chem* 2005; **51** (1): 244–7.
14 Simundic AM, Nikolac N, Ivankovic V *et al.* Comparison of visual *vs.* automated detection of lipemic, icteric and hemolyzed specimens: can we rely on a human eye? *Clin Chem Lab Med* 2009; **47** (11): 1361–5.
15 Lippi G, Blanckaert N, Bonini P *et al.* Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. *Clin Chem Lab Med* 2008; **46** (6): 764–72.
16 Stark A, Jones BA, Chapman D *et al.* Clinical laboratory specimen rejection – association with the site of patient care and patients' characteristics: findings from a single health care organization. *Arch Pathol Lab Med* 2007; **131** (4): 588–92.
17 Alsina MJ, Alvarez V, Barba N *et al*. Preanalytical quality control program – an overview of results (2001-2005 summary). *Clin Chem Lab Med* 2008; **46** (6): 849–54.
18 Dale JC, Novis DA. Outpatient phlebotomy success and reasons for specimen rejection. *Arch Pathol Lab Med* 2002; **126** (4): 416–9.
19 Guimaraes AC, Wolfart M, Brisolara MLL, Dani C. Causes of rejection of blood samples handled in the clinical laboratory of a University Hospital in Porto Alegre. *Clin Biochem* 2012; **45** (1–2): 123–6.
20 Söderberg J, Jonsson PA, Wallin O, Grankvist K, Hultdin J. Haemolysis index – an estimate of preanalytical quality in primary health care. *Clin Chem Lab Med* 2009; **47** (8): 940–4.
21 Francis DL, Prabhakar S, Sanderson SO. A quality initiative to decrease pathology specimen labeling errors using radiofrequency identification in a high volume endoscopy center. *Am J Gastroenterol* 2009; **104** (4): 972–5.
22 Sodi R, Darn SM, Stott A. Pneumatic tube system induced haemolysis: assessing sample type susceptibility to haemolysis. *Ann Clin Biochem* 2004; **41** (Pt 3): 237–40.
23 Lowe G, Stike R, Pollack M *et al.* Nursing blood specimen collection techniques and hemolysis rates in an emergency department: analysis of venipuncture versus intravenous catheter collection techniques. *J Emerg Nurs* 2008; **34** (1): 26–32.
24 Bush RA, Mueller T, Sumwalt B, Cox SA, Hilfiker ML. Assessing pediatric trauma specimen integrity. *Clin Lab Sci* 2010; **23** (4): 219–22.