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Suitability of Frozen Serum Stored in Gel Separator Primary Sampling Tubes for Serological Testing

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The suitability of frozen serum after storage in primary sampling tubes with a gel separator for serological enzyme-linked immunosorbent assay testing (hepatitis B virus surface antigen [HBs Ag], anti-HBs Ag, anti-*Toxoplasma gondii* immunoglobulin G [IgG], anti-rubella virus IgG, anti-cytomegalovirus IgM, and anti-Epstein-Barr virus IgM) was evaluated for 375 samples. No difference was found among test results using fresh or stored frozen serum

Serum separator tubes were introduced into laboratories approximately 25 years ago and since have gained widespread acceptance due to the advantage of a barrier gel that facilitates rapid separation of serum from cells. Use of these tubes makes drawing blood easier, facilitates blood clotting and rapid separation of serum, reduces centrifugation time (they withstand higher centrifugation speed), and avoids transfer of serum to new tubes, contributing to improved quality of the preanalytical phase (8, 9).

In general, serum for serology tests can be stored at 2 to 8°C for a few days before testing (1, 5, 10, 12). Longer storage can be necessary, however, for confirmatory tests, quality control, seroepidemiological studies, demonstration of a significant increase in the titer of antibodies in paired acute and convalescent-phase sera, or other tests (6). For prolonged storage, the separated serum should be kept frozen in a new tube at −20°C or lower, avoiding repeated freeze-thaw cycles (5, 13). Here we report the results of an evaluation of the suitability of sera for serological testing when preserved frozen in serum gel separator primary sample collection tubes.

Samples. We analyzed 375 sera received in our laboratory for serological studies. Blood (5 ml) was collected into a polyethylene terephthalate serum-gel-separator tube (Venojet II plastic vacuum tube; Terumo Europe, Leuven, Belgium). Within 4 h of the blood draw, tubes were centrifuged at 1,500 × *g* for 15 min, and initial testing was performed on the same day they were processed for storage.

After initial testing, gel separator tubes (containing the remaining serum, the gel, and the cell blood layer) were stored at −20°C, tightly capped with parafilm. In addition, from 140 out of the 375 samples, 0.5 ml of the serum was transferred to a polypropylene tube, which was also stored at −20°C. After 5 to 6 months' storage, the samples were thawed at room temperature and gently mixed, and serological analytes were determined again.

Serological tests. The sera studied included positive and negative samples (Table 1) for hepatitis B surface antigen (HBs Ag), antibody to HBs Ag (anti-HBs Ag), anti-hepatitis C virus antibodies (anti-HCV Ag), anti-*Toxoplasma gondii* immunoglobulin G (IgG) antibodies (anti-Toxo IgG), anti-rubella virus IgG antibodies (anti-Rub IgG), anti-cytomegalovirus IgM antibodies (anti-CMV IgM), and anti-Epstein-Barr virus (VCA, EBNA, and EA antigens) IgM antibodies (anti-EBV IgM). All samples were tested using enzyme-linked immunosorbent assay (ELISA) microplate assays. Enzygnost tests (Dade-Bhering, Marburg, Germany) were used for all, with the exception of anti-HCV Ag, which was assayed using the Ortho HCV 3.0 ELISA test system (Ortho-Clinical Diagnostics, Inc., Raritan, N.J.). All testing was done in accordance with manufacturers' guidelines. Positive and negative controls were performed on each batch of tests. Serum samples (whether fresh or thawed) were directly handled using a 150 Genesis robotic sample processor (Tecan AG, Hombrechtikon, Switzerland), and further processing was performed in a Bhering ELISA processor III (Dade-Bhering).

The initial evaluation (step 1) of the suitability of frozen serum preserved in gel separator tubes for serological testing was carried out in 235 sera, comparing the qualitative results of the tests on the day of collection and after storage. Afterward (step 2), 140 sera, 10 positive and 10 negative for each sero-

TABLE 1. Tests and number of sera used for evaluation of the suitability of frozen serum preserved in gel separator tubes for serological testing

Serological test[a]	Sera studied qualitatively (step 1)		Sera studied qualitatively and quantitatively (step 2)	
	No. positive	No. negative	No. positive	No. negative
HBs Ag	18	25	10	10
Anti-HBs Ag	29	25	10	10
Anti HCV Ag	22	25	10	10
Anti Toxo IgG	11	11	10	10
Anti-Rub IgG	22	15	10	10
Anti CMV IgM	6	6	10	10
Anti EBV IgM	10	10	10	10
Total	118	117	70	70

[a] For abbreviations, see the text.

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TABLE 2. Comparison of absorbance values of 140 frozen sera for seven serological ELISA tests after five months' storage in gel separator tubes and in polypropylene tubes[a]

Test	Results for Positive sera (n = 70, 10 for each test)					Results for negative sera (n = 70, 10 for each test)				
	Positive cutoff[b]	Absorbance range		Δ Absorbance[c]	P^d value	Negative cutoff[e]	Absorbance range		Δ Absorbance	P value
		Gel separator tube	Polypropylene tube				Gel separator tube	Polypropylene tube		
HBs Ag	0.088	0.532–3.847	0.430–3.740	0.029 ± 0.3	0.75	0.088	0.025–0.041	0.024–0.042	0.001 ± 0.07	>0.9
Anti-HBs Ag	0.125	0.342–3.413	0.401–3.433	0.019 ± 0.1	0.85	0.125	0.038–0.103	0.040–0.102	0.003 ± 0.09	>0.9
Anti HCV Ag	0.379	0.693–3.734	0.758–3.891	0.029 ± 0.3	0.75	0.379	0.022–0.043	0.026–0.045	0.001 ± 0.08	>0.9
Anti Toxo IgG	0.127	0.410–1.815	0.476–1.760	0.067 ± 0.3	0.50	0.047	0.020–0.035	0.014–0.039	0.002 ± 0.08	>0.9
Anti-Rub IgG	0.200	0.658–2.118	0.551–2.239	0.050 ± 0.3	0.65	0.100	0.004–0.032	0.006–0.033	0.003 ± 0.07	>0.9
Anti CMV IgM	0.200	0.241–1.034	0.212–1.260	0.007 ± 0.3	>0.9	0.100	0.003–0.072	0.002–0.084	0.004 ± 0.12	>0.9
Anti EBV IgM	0.170	0.325–0.725	0.346–0.817	0.030 ± 0.3	0.7	0.100	0.001–0.064	0.003–0.070	0.001 ± 0.09	>0.9

[a] Each test was carried out in a single run and in a single microplate.
[b] Minimum value of absorbance required to consider a test result positive.
[c] Mean ± standard deviation of differences of absorbances between sera stored frozen in gel separator tubes and polypropylene tubes.
[d] Probability paired t test.
[e] Maximum value of absorbance required to consider a test negative.

logical test, were studied. We compared not only the qualitative results of the tests but also the quantitative results (absorbance readings) obtained from sera stored frozen in gel separator tubes and in polypropylene tubes. For each analyte, sera kept frozen in gel separator tubes and the fraction kept frozen in polypropylene tubes (in total, 40 samples) were thawed and analyzed in a run and in a single microplate to avoid interassay variability.

There was total agreement between all qualitative results for the 375 sera (Table 1, steps 1 and 2) that were tested on the day of collection and after being stored frozen in gel separator tubes. No misclassification was noticed in any serological test using sera stored frozen either in polypropylene tubes or in gel separator tubes. No significant difference (paired t test) was found among the absorbance readings obtained with the 140 sera preserved frozen in propylene tubes and in gel separator tubes (Table 2).

After thawing, eight (2.1%) out of the 375 sera kept frozen in gel separator tubes showed a light red color, indicating passage of hemoglobin through the gel. Of these hemolyzed samples, two were positive for HBs Ag, one was positive for anti-HCV Ag, one was positive for anti-Toxo IgG, two were negative for Anti-Rub IgG, one was negative for HBs Ag, and one was negative for anti-HBs Ag. Qualitative results from all eight hemolyzed sera (gel separator tubes) showed no misclassification when they were compared with nonhemolyzed sera, either fresh or preserved frozen in a polypropylene tube. Three out of these eight sera (one negative for HBs Ag, one negative for anti-HBs Ag, and one positive for anti-Toxo IgG) were studied quantitatively (step 2). No significant change in absorbance values in the ELISA tests was noticed between the frozen serum stored either in the gel separator tube (slightly hemolyzed) or in the polypropylene tube. The difference between the absorbance readings for each of these sera pairs was always within the standard deviation for the differences of absorbances in the test.

In general, gel separator tubes are suitable for collection and short-term storage of blood for commonly ordered laboratory tests (4). But due to absorption of drugs by the gel, a significant reduction in the concentration of therapeutic drugs when blood is kept in these tubes has been reported (3, 9). We have not, however, found significant differences between the Ig concentrations (absorbance readings in the ELISA tests) in serum samples kept frozen in polypropylene tubes and those in samples kept in gel separator tubes.

A point of concern when dealing with clinical chemistry analytes is hemolysis. If this is present it can cause spurious tests results, and estimation of some analytes (e.g., hydroxybutyrate dehydrogenase, aspartate transaminase, creatine, bicarbonate, and potassium) are not valid (2, 7, 11). It has also been reported that hemolyzed serum may not be suitable for serological testing (5). In this study results were not affected because of passage of some hemoglobin through the gel. Nevertheless, since this happened in only a few sera, further work is necessary before any general conclusion can be drawn on this point.

The purpose of quality control is to prevent as many errors as possible and to detect those which do occur, but according to Murphy's Law, if anything can go wrong, it will (13). Handling of sera for storage not only is prone to technical and clerical errors (labeling of tubes and keeping the inventory) but also can lead to serious biological risks, since it involves handling potentially highly infectious material (hepatitis B and C virus, human immunodeficiency virus, and other potential pathogens). The finding that serum samples for ELISA tests can be stored at −20°C in primary tubes with a serum gel separator can avoid potential errors in sample identification and can decrease the workload associated with serum storage. This can also minimize the risks of biohazard associated with the transfer of sera to new tubes. In conclusion, we have found that stowing frozen centrifuged blood in the primary collection tube with a gel separator barrier could be an alternative to the transfer of serum to new tubes for storage. Further studies will be necessary, however, to prove that this approach is generally valid for serological tests.

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