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|  | Principle |
|  | To determine the optimal time of centrifugation for agglutination tests. |
|  | Scope and Related Policies |
|  | 2.1 | The speed of rotation and the timing device of a centrifuge shall be calibrated on installation, following servicing and maintained as per manufacturer’s recommendation’s 9.1 or every six months when not specified. |
|  | 2.2 | The optimal time of centrifugation of each serologic centrifuge should be determined upon receipt, after repair and yearly thereafter. |
|  | 2.3 | Records should be retained for the life of the instrument plus five years.9.1 |
|  | 2.4 | A label indicating date calibration performed and when next calibration date is due must be applied to the equipment.9.2 |
|  | Specimens – N/A |
|  | Materials |
|  | **Equipment:** Serologic centrifuge  Operator manual for centrifuge Stopwatch**Supplies:** Test tubes Functional Calibration of Serologic Centrifuges  (QCA.007F)**Reagents:** Antisera (may be outdated)  Antigen positive cells (corresponding to the antibody present  in the QC antisera) Antigen negative cells (corresponding to the antibody present  in the QC antisera) Potentiator solution (PEG or LISS) Anti-IgG Saline Plasma |
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|  | Quality Control – N/A |
|  | Procedure |
| * 1. Upon receipt (and scheduled calibration) or after repair of a serologic centrifuge:
 | * + 1. Install the instrument as per the manufacturer’s instructions. Ensure that the operator manual is available.
 |
| * + 1. Check the timer. See Procedural Notes 8.1.
 | * + - 1. Turn the timer on for 10 seconds and start the stopwatch immediately.
 |
| * + - 1. As soon as the centrifuge starts decelerating, stop the stopwatch. See Procedural Notes 8.2.
 |
| * + - 1. Compare the time of centrifugation with the time on a stopwatch.
 |
| * + - 1. Record the time on form QCA.007F.
 |
| * + - 1. Repeat steps 6.1.2.1 – 6.1.2.4 with the timer settings at 15, 20, 30 and 45 seconds.
 |
| * + - 1. The timing device must be checked and maintained as per manufacturer’s recommendations or every 6 months when not specified.
 |
| * + 1. Perform the functional calibration for optimal time of centrifugation for agglutination tests.
 | * + - 1. Prepare a diluted antisera:
* Prepare a serial, 2 fold dilution of antiserum (e.g., anti-C or anti-D) up to 512. See Procedural Notes 8.3. Diluting solution should be plasma.
* Test each dilution against a known positive cell using the routine antiglobulin method in your laboratory (e.g., LISS, PEG or SAL).
* Choose the dilution that reacts as a strong grade 1 reaction with the positive cell. This is the “optimal dilution”.
* Write the “optimal dilution” on QCA.007F. Discard the titration tubes.
* Dilute the commercial antiserum with the same solution used for the titration to obtain a final volume of 5 mL.
 |
| * + - 1. Label 10 tubes as follows: 10+, 10-, 15+, 15-, 20+, 20- 30+, 30-, 45+, 45-.
 |
| * + - 1. Add 2 drops of the diluted antisera to each tube.
 |
| * + - 1. Add 1 drop of “antigen” positive red cells to tubes 10+, 15+, 20+, 30+ and 45+.
 |
| * + - 1. Add 1 drop of “antigen” negative red cells to tubes 10-, 15-, 20-, 30- and 45-.
 |
| * + - 1. Add 2 drops of potentiating solution to each tube. Omit if performing saline IAT.
 |
| * + - 1. Incubate the tubes for 15 minutes at 37°C (30-60 minutes if performing saline IAT).
 |
| * + - 1. After incubation, wash 4 times.
 |
| * + - 1. Remove the tubes from the cell washer and set aside, except tubes 10+ and 10-.
 |
| * + - 1. Immediately before centrifugation, add 2 drops of anti-IgG to tubes 10+ and 10-.
 |
| * + - 1. Centrifuge the tubes for 10 seconds in the equipment (i.e. centrifuge or cell washer) being calibrated.
 |
| * + - 1. Remove the tubes from the centrifuge or cell washer and check the appearance of the:
* supernatant (is it clear?)
* red cell button (is it clearly delineated and the periphery sharply defined, not fuzzy?)
 |
| * + - 1. Record the results on QCA.007F.
 |
| * + - 1. Resuspend cells and observe both tubes for the following:
* is the cell button easily resuspended?
* strength of agglutination (grade 1 for the positive test)
 |
| * + - 1. Record observations and results on form QCA.007F.
 |
| * + - 1. Repeat steps 6.1.3.10 and 6.1.3.15 for each set of tubes 15, 20, 30 and 45 varying the centrifugation time to the time written on the tube.
 |
| * + 1. Based on the results, select the optimal time of centrifugation for agglutination.
 |
| * + 1. The optimal time of centrifugation is the least amount of time required to fulfil the following criteria:
* Supernatant fluid is clear
* Red cell button is clearly delineated and the periphery is sharply defined, not fuzzy
* Red cell button is easily resuspended
* Strength of the reaction is as expected (i.e., grade 1 and negative)
 |
| * + 1. Document the optimal time of centrifugation on QCA.007F and on the appropriate cell washer or serologic centrifuge.
 |
| * 1. Once a year:
 | * + 1. Perform a functional calibration for optimal time of centrifugation for agglutination tests. See steps 6.1.3 to 6.1.6.
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|  | Reporting – N/A |
|  | Procedural Notes |
|  | 8.1 | Timer/Stopwatch Validation |
|  |  | 8.1.1 | Take a timer/stopwatch and call NRC for accurate time (1-613-745-1576). Ensure that the timer/stopwatch you are using to test the other timers matches the NRC time (NRC will give 30 seconds elapsed time). |
|  |  | 8.1.2 | To test other timers in laboratory: one minute shall be the minimum time tested. If timers appear not to be keeping proper time inform Operations Manager or Charge Technologist. |
|  | 8.2 | The centrifugation time includes the time of acceleration but not deceleration. |
|  | 8.3 | The dilution may vary depending on the concentration of the antibody in the antisera. |
|  | References |
|  | 9.1 | Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine, 3.1.4, 3.1.5, 3.4.3.1, 6.6.9 Appendix A. |
|  | 9.2 | IQMH Requirements and Guidance Information, version 6 December 2013; IV |
|  | 9.3 | Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 972-973. |

# Revision History

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| **Revision Date** | **Summary of Revision** |
| September 1, 2015 | * Revised name of manual
* Revised sections 2.0 & 6.0
* Updated list of references to include most recent editions
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