

Capture-R® Ready-Screen® (Pooled Cells)

Solid Phase System for the Detection of Unexpected IgG Antibodies to Red Cells

Rx ONLY

346-14



Immucor, Inc.
3130 Gateway Drive
Norcross, GA 30071 USA
Immucor Medizinische Diagnostik GmbH
Robert-Bosch-Strasse 32
63303 Dreieich, GERMANY

EC REP

IMMUCOR®

Intended Use:

Capture-R® Ready-Screen® (Pooled Cells) is intended for use in the detection of unexpected IgG antibodies to red blood cells by manual, semiautomated or automated solid phase red blood cell adherence methods.

Summary of the Test:

Unexpected antibodies are found in the sera of 0.3 to 3% of donor and patient populations.¹⁻³ Many antibodies are of clinical importance since they may cause decreased red blood cell survival as the result of hemolytic transfusion reactions, hemolytic disease of the newborn or autoimmune hemolytic anemia. In vitro antibody detection (screening) tests are employed to reveal the presence of these antibodies in patient and donor sera. Selected red blood cells, such as those provided as Capture-R Ready-Screen, are incubated with test sera or plasma under conditions that will facilitate antibody detection.⁴ Capture-R Ready-Screen (Pooled Cells) is not recommended for pretransfusion tests done in lieu of a major crossmatch to detect unexpected antibodies in patients' samples.

Principle of the Test:

Capture-R Ready-Screen is a modified solid phase antibody detection systems based on the procedures of Plapp et al⁵ and Juji et al.⁶ Membranes of red blood cells have been bound to and dried on the surfaces of polystyrene microwells. The membrane antigens are used to capture red blood cell-specific antibodies from patient or donor sera or plasmas. Following a brief incubation period, unbound residual immunoglobulins are rinsed from the wells and replaced with a suspension of anti-IgG-coated indicator red blood cells. Centrifugation brings the indicator red cells in contact with antibodies bound to the reagent red blood cell membranes. In the case of a positive test, the migration of the indicator red blood cells to the bottom of the wells is impeded as anti-IgG-IgG complexes are formed on the surface of the immobilized reagent layer. As a consequence of antibody bridging, the indicator red cells adhere to the screening cells as a second immobilized layer. In the absence of detectable antigen-antibody interactions (negative test), the indicator red blood cells will not be impeded during their migration and will pellet to the bottom of the wells as tightly agglutinated red cell buttons.

Reagents:

- Capture-R Ready-Screen (Pooled Cells)** consisting of 1 x 8 strips carrying the bound and dried red blood cell membranes prepared from a pool of two group O donors. Twelve 1 x 8 strips are packaged with a support frame and enclosed in a foil pouch to which a desiccant and moisture indicator have been added. Each strip is ready to be used as supplied. Strips can be used singly or in multiples. Store the strips at 1-30 C (under refrigeration or at room temperature) when not in use. If the humidity indicator enclosed within a pouch shows the presence of moisture (by the humidity indicator turning from blue to pink), the strips should not be used. Unused strips, desiccant and moisture indicator should be carefully resealed within the foil pouch to prevent exposure to moisture that can destroy the red blood cell membranes. Strips within resealed pouches should not be used if the humidity indicator shows the presence of moisture. **Strips removed from pouches should be used within eight hours.**
- Master List:** provided with each lot of Capture-R Ready-Screen indicates the code and antigenic composition of each donor whose red blood cells are used to prepare the dried reagent monolayers.

Adjunct Reagents to Capture Test Wells:

- Capture LISS:** a low ionic strength solution containing glycine, bromocresol purple dye and the preservative sodium azide (0.1%)*. Store at 1-10 C.

- Capture-R Ready Indicator Red Cells:** a suspension of red blood cells coated with murine monoclonal anti-human IgG molecules. The reagent is suspended in a buffered preservative solution to which chloramphenicol (0.25 mg/mL), neomycin sulfate (0.1 mg/mL) and gentamycin sulfate (0.05 mg/mL) have been added as preservatives. Store at 1-10 C.

- Capture-R Positive Control Serum (Weak):** contains antibodies to red blood cells. Sodium azide (0.1%) is added as a preservative*. Store at 1-10 C.

- Capture-R Negative Control Serum:** contains no antibodies to red blood cells. Sodium azide (0.1%) is added as a preservative*. Store at 1-10 C.

NOTE: The in-date components (Capture-R Ready-Screen wells, Capture-R Ready Indicator Red Cells, Capture LISS and Capture-R Control Sera) used to perform Capture-R Ready-Screen assays can be used interchangeably with other component lots, provided the components are within their dating periods. NOTE: Master Lists are lot specific.

Precautions:

- For in vitro diagnostic use.
-



This reagent contains 0.1% sodium azide. Warning: H302 Harmful if swallowed.

Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into the sink, flush with a large volume of water to prevent azide build-up.

- All Capture-R Ready-Screen reagents must be brought to 18-30 C before testing.
- Capture-R Ready Indicator Red Cells must be suspended before use by gently inverting each vial several times. It is normal for Capture-R Ready Indicator Red Cells to aggregate slightly during 1-10 C storage. Capture-R Ready Indicator Red Cells should not be used if the red blood cells darken from red to brown, if there is hemolysis, or if the cells fail to perform properly in positive and negative control tests. Slight hemolysis may occur with age.
- Turbidity of Capture LISS and Capture Control Reagents may be an indication of microbial contamination. Reagents that are contaminated should not be used.
- Do not use reagents beyond their expiration dates. Leaking vials should not be used.
- The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).
- Handle and dispose of reagent as if potentially infectious.

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.

Specimen Collection and Preparation:

Plasma or serum: Draw a blood specimen using an acceptable phlebotomy technique. Fresh serum or plasma (EDTA, ACD, CPD, CPDA-1, CP2D) may be used in this assay. All testing should be performed as soon as possible following collection to minimize the chance of false-positive or false-negative reactions due to improper storage or contamination of the specimen. Specimens that cannot be tested within 24 hours should be stored at 1-10 C as soon as possible. Alternatively, specimens can be separated from red blood cells and stored frozen. Weakly reactive antibodies may deteriorate and become undetectable in specimens stored at room temperature for several days before testing or in specimens stored for prolonged periods at 1-10 C. Do not use specimens

Key:

Underline = Addition or significant change; ▲ = Deletion of text

drawn into tubes containing neutral gel separators. False-positive results may occur with such specimens.

Procedure:

A. Materials Provided:

1. Capture-R Ready-Screen (Pooled Cells) Microwells in resealable foil pouches

B. Additional Capture Materials Required:

1. Capture LISS in dropper vials
2. Capture-R Ready Indicator Red Cells in dropper vials
3. Capture-R Positive Control Serum (Weak) in dropper vials
4. Capture-R Negative Control Serum in dropper vials

C. Additional materials required for manual and semi-automated method:

1. Donor or patient serum or plasma
2. Marking pens
3. Transfer pipettes or pipetting system*
4. Centrifuge* with rotor capable of accommodating 1 x 8 and 2 x 8 strips of wells
5. 37 C heat block or dry heat incubator
6. Phosphate-buffered saline, (approximately 15 mM), pH 6.5-7.5
7. Washing device* or wide port saline wash bottle or manual dispensing manifold
8. Dispensing manifold or pipettors designed for microplates
9. Blank strips of wells for balance
10. Microplate reader* (optional)

D. Additional materials required for automated method:

1. NEO Iris* (as applicable)

For testing with automated instrumentation, refer to the instructions provided in the instrument operator manual.

* It is the users responsibility to validate an accessory device (either listed or otherwise) for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

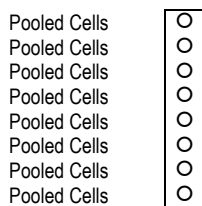
Test Method:

Manual or Semi-automated Methods:

1. Bring all Capture reagents and specimens to 18-30 C before testing.
2. Remove one Capture-R Ready-Screen Strip from its protective pouch. Inspect the humidity indicator enclosed in the pouch. If the humidity indicator shows the presence of moisture, none of the strips within the pouch should be used. In the absence of signs of moisture, return unused strips, desiccant and humidity indicator to the pouch and carefully reseal the pouch.
3. Check the bottom tab of the strip. Do not use the strip if it is not imprinted to show the test identification. The arrangement of Reagent Red Blood Cells is shown in Fig. 1.
4. Place the strip in a frame holder. Note: the strip will only fit into the holder in the correct orientation.
5. Add 2 drops (100 +/- 10 uL) of Capture LISS to each test and control well.
6. Addition of Controls and patient or donor specimens to Capture-R Ready-Screen (Pooled Cells) Strip Wells:
 1. Add 1 drop (50 ± 5 uL) of Capture-R Positive Control Serum (Weak) to one well.
 2. Add 1 drop (50 ± 5 uL) of Capture-R Negative Control Serum to another well.
 3. Add 1 drop (50 ± 5 uL) of the test serum/plasma to separate wells.

NOTE: If more than one strip is needed to test patient or donor specimens, additional strips may be used without including control reagents, up to one full frame holder. Note: At least one set of control reagents (Weak Positive and Negative Controls) should be included with each test run or for each completed frame holder to monitor that the strips have been centrifuged and/or washed properly.

Fig 1. Capture-R Ready-Screen (Pooled Cells) Strip



7. Incubate the strips at 36-38 C for no less than 15 minutes and no longer than 60 minutes. Add 5 minutes to the incubation period if a dry heat incubator is used.
8. Decant or aspirate the sample-LISS mixture from the wells and wash the wells using a manual or automated washing technique.
 - a. Manual Washing Technique
 - i. Decant fluid from the wells.
 - ii. Fill the wells of the strip with saline dispensed from a multichannel dispenser or manifold designed for microplates. Alternatively, a saline

wash bottle can be used to dispense the saline. Saline should not be added with excessive force since this may cause the red cell monolayer to disengage from the plate.

- iii. Decant the wells thoroughly by manually inverting the strip wells over a sink or waste receptacle and with several rapid, sharp motions, dumping the saline from the wells.
 - iv. Wash the wells a minimum of six times with saline.
- b. Automated Washing Technique
 - i. Prime the instrument and intake lines with isotonic saline according to the instrument manufacturer's directions.

NOTE: When using strips on automated washers, the strip well holder (frame) must be full of test and/or empty strips.

- ii. To remove sample from the wells, aspirate the contents of each well with a vacuum device.
- iii. Sequential aspiration/dispense washers: Wash each well a minimum of three times by filling each well with at least 300 uL of saline and then aspirating the well contents with a vacuum device. Consult the instrument manufacturer's operating manual for a description of the proper use of the microplate washing device. After the first three washes, rotate the strip 180 degrees and wash a minimum of three more times. In the event that one of the dispensing or aspirating probes of the washer has become clogged, this increases the likelihood that all test wells will be washed sufficiently.

NOTE: The automated washing device must be adjusted such that approximately 4-8 uL of saline remains in each well after aspiration. Wells should not be aspirated until they are dry.

9. Add 1 drop (50 +/- 5 uL) of Capture-R Ready Indicator Red Cells to each of the wells.
10. Immediately centrifuge the strip for 1-3 minutes at 450-600xg.

NOTE: The g forces and time given are approximations of forces required to produce the desired degree of adherence. The appropriate g forces (or rpms) and centrifuge time must be determined individually for each centrifuge used.

11. Place the strip on an illuminated surface and examine for adherence or the absence of Indicator Red Cell adherence. For test results to be considered valid, the following reactions must be obtained with the Capture-R Control Sera each time a plate is tested.
 - Positive Control (Weak) = adherence of Capture-R Ready Indicator Red cells to all or part of the reaction surface.
 - Negative Control Serum = button of Capture-R Ready Indicator Red cells at the bottom of the test wells.

If the correct reactions are not obtained with the Capture-R Control Sera, test reactions may be invalid and the tests of that run must be repeated.

Automated Method:

For testing with automated instrumentation, refer to the instructions provided in the instrument operator manual.

Stability of Reaction:

Following centrifugation, manual and semiautomatic tests can be read immediately. Wells can be covered following centrifugation to prevent evaporation, stored at 1-10 C, and read or reread manually up to 2 days following testing.

Automated instrumentation reads results at test completion and stores results for reporting at the completion of the batch operation.

Quality Control:

Daily Quality Control of all Capture-R Ready-Screen components is built into the test systems by the inclusion of the Capture-R Positive and Negative Controls. These Controls should be included with each centrifugation run, whether that run consists of one strip, or more than one strip, to ensure that neither technical errors (e.g. improper washing or centrifugation), nor reagent failures, have occurred. Continued failure of the Controls to perform properly on repeat testing may indicate that one or more of the Capture-R Ready-Screen test reagents have deteriorated, or that tests are consistently being performed incorrectly.

For microplate testing with automated instrumentation, refer to the instructions provided in the instrument operator manual.

Interpretation of Results:

Negative test: button of Capture-R Ready Indicator at the bottom of the test well with no area of adherence.

Positive test: adherence of Capture-R Ready Indicator Red Cells to part or all of reaction surface.

Key:

Underline = Addition or significant change; ▲ = Deletion of text

Instrumentation automatically interprets test results.

Antibodies that are detected using Capture-R Ready-Screen can be identified using either Capture-R Ready-ID, Capture-R Ready-ID Extend I and II or Capture-R Select, a solid phase system that can be used with reagent red blood cells of all manufacturers.

Limitations:

1. Erroneous test results can occur from bacterial or chemical contamination of test materials, inadequate incubation periods, improper centrifugation, inadequate washing of test wells, or omission of test reagents or steps.
2. Contamination of Capture-R Ready Indicator Red Cells with IgG-containing serum or plasma proteins will neutralize the anti-IgG component of the Capture-R Ready Indicator Red blood cells, leading to falsely negative test results. Failure of the Capture-R Positive Control is an indication of indicator red cell neutralization in manual or semiautomated testing.
3. Overcentrifugation of the tests, following addition of the Capture-R Ready Indicator Red Cells, may result in falsely negative or doubtful positive reactions due to the collapse of the adherent indicator layer. Undercentrifugation will lead to falsely positive results.
4. Examples of pure IgG4 subclass antibodies may not be detected by the Capture-R Ready Indicator Red Cell reagent. Note, however that pure IgG4 antibodies are very uncommon.
5. The deceleration parameters of the centrifuge in use may effect the type of reactions obtained at the end of the assay. Failure to apply the braking mechanism in units with long deceleration times may result in falsely negative reactions. Conversely, braking of centrifuges with short deceleration times may also cause erroneous test results. It is the users responsibility to evaluate centrifuge performance before use to identify optimum spin speeds, spin times and acceleration/deceleration settings. The results of the performance evaluation should be maintained as part of the laboratory's records for review by regulatory agencies.
6. Serum or plasma specimens obtained from tubes containing neutral gel separators may produce falsely positive results in antibody screening tests. Tubes with gel separators are not designed for blood bank use.
7. The reactivity of Capture-R Ready-Screen reagent red blood cells may diminish over the dating period. The rate at which antigen reactivity is lost is partially dependent on the individual donor characteristics that are neither controlled or predicted by the manufacturer.
8. Addition of Capture-R Ready Indicator Red Cells in excess of amounts described in this insert may result in falsely negative or doubtful test reactions.
9. Addition of too few indicator red cells, as might occur with improper mixing of the reagent or through hemolysis of the red blood cells, will cause weak falsely positive results. Indicator red cells that are colder than 18 C when used will cause weak false-positive results.
10. Low ionic strength solutions (LISS) have been shown to enhance many antigen-antibody interactions. However, sera may be encountered that contain antibodies that are not optimally reactive in LISS test systems including the Capture-R Ready-Screen assay.
11. Antibodies such as anti-M, -P₁, -Le^a and -Le^b frequently react in tube hemagglutination tests at the room temperature phase of testing rather than at 37 C or at the antiglobulin phase. Some workers have interpreted this to mean that the antibodies were composed mostly of saline-reactive IgM molecules. Some examples of these antibodies may be detected in Capture-R assays, even though the test system is designed primarily for the detection of IgG because they contain an IgG component. Others may be detected, not because they are IgG in nature, but because the Indicator Red Cells carry the antigen toward which the IgM antibody is directed. Some IgM antibodies have been found to link Indicator Red Cells to immobilized red blood cell monolayers by binding to antigens on both. Thus, examples of anti-M, -Le^a, -Le^b, -P₁, etc that are detected in Capture-R tests should not be assumed to contain an IgG component without further study. These specificities are regarded as insignificant in most clinical situations. Examples of these antibodies detected in Capture-R tests are not necessarily more significant than examples that fail to react. Specificities of presumed significance, that are wholly IgM in nature (ie, IgM anti-K or IgM anti-E) may fail to react in this assay.
12. Some IgG antibodies have been shown to react poorly in solid phase red blood cell adherence assays. These include examples of antibodies to Bg^a, Bg^b, Kn^a, Cs^a, Yk^a, JMH, McC^a, Ch and Rg. ^{7,8} Weak examples of clinically relevant antibodies, may fail to react by Capture-R Ready-Screen, even though the antibodies are detected by an alternative technique. Passively administered anti-D may fail to react with Capture-R Ready-Screen. **NO ONE TEST METHOD IS CAPABLE OF DETECTING ALL ANTIBODIES.**

13. Antibody screening tests employing pooled reagent red blood cells will not be as sensitive as those incorporating the red blood cells of unpooled single donors. Pooled reagent red blood cells should not be used when the antiglobulin antibody screen is performed in place of the antiglobulin crossmatch.
14. The red blood cells used to prepare this reagent can carry antigens that are not defined by the manufacturer. Therefore, it is possible to obtain positive reactions with this product that do not match the profiles of any antigens shown on the Master List.
15. The genetic background of donors of Reagent Red Cells with phenotypes such as Fy(a+b-), Fy(a-b+), Jk(a+b-), Jk(a-b+), M+N-, M-N+, S+s- or S-s+ is not known. Such red blood cells are assumed to be from genetic homozygotes, but in fact, could have been collected from persons who are genetically heterozygous for the encoding genes.⁴ No serological tests have been performed to demonstrate the red blood cells of apparent homozygotes used to prepare Capture-R Ready-Screen carry a double dose of the appropriate antigens.⁸
16. Negative reactions will be obtained if the test specimens contains antibodies present in concentrations too low to be detected by the test methods employed.⁸
17. Reactions between an antibody and its antigen may be weakened if acidic or unbuffered saline is used to wash test wells prior to the addition of Indicator Red Cells. Best results will be obtained with saline buffered to pH 6.5-7.5⁹
18. Incorrect results may be obtained in Capture-R Ready-Screen assays if testing personnel are not adequately trained in proper test performance. A laboratory that institutes Capture-R Ready-Screen technology should have a program that will properly train personnel. After personnel have received sufficient training, but before existing antibody detection techniques are replaced with Capture-R Ready-Screen, the laboratory should perform parallel evaluations with Capture-R Ready-Screen and the house method (using a large battery of known positive and negative samples) to document that the appropriate results can be obtained.

Specific Performance Characteristics:

Clinical evaluations of over 7,000 samples performed by five separate laboratories demonstrated that the Capture-R Ready-Screen assays were capable of detecting clinically important IgG antibodies to red blood cells. Each laboratory involved in the study used plasma or serum specimens that were tested by a reference hemagglutination assay in parallel. Capture-R Ready-Screen has been shown to detect most clinically significant antibodies of the IgG subclass. IgG antibody specificities not readily detected in these studies are listed in the LIMITATIONS section of this insert. Some patient and donor specimens were evaluated that reacted by Capture-R Ready-Screen, but were nonreactive by reference hemagglutination techniques. Most of these specimens were shown to contain solid-phase only autoantibodies.

The antiglobulin coating of the Capture-R Ready Indicator Red Cells is evaluated in potency tests with anti-D and anti-Fy^a.

Prior to the manufacture of Ready-Screen, the red blood cells of each donor are tested by two independent laboratories using no less than two donor sources of antibody (except where precluded by the rarity of the antisera) to confirm the presence or absence of all blood group antigens specified on the Master List. All red blood cells are tested and shown to have a negative direct antiglobulin test using polyspecific anti-human globulin.

Performance Characteristics on NEO Iris:

Method comparison studies were performed at three external clinical sites, including transfusion services and donor centers. Immucor, Inc., as the manufacturer, was a fourth site. Specimens were tested on NEO Iris and Galileo Neo. Test results were evaluated for agreement between analyzers. Combined results from all sites are summarized in the following tables:

Note: Agreement between methods does not indicate which method is correct.

Random Samples N=1857		Galileo Neo / Manual			
		Positive	Negative		
NEO Iris	Positive	10	3	Positive % Agreement	100.0%
				PPA (95% Lower Bound One-Sided CI)	74.1%*
	Negative	0	1844	Negative % Agreement	99.8%
				NPA (95% Lower Bound One-Sided CI)	99.6%

Key:
Underline = Addition or significant change; ▲ = Deletion of text

*PPA lower 95% CI did not meet 99% due to the low frequency of antibody positive samples in the population. Results are for North America Market assays. Discordant and equivocal samples were further tested by manual antibody screen method. Additional well-characterized antibody positive samples were further tested, see results in table below.

Well-characterized Samples N=283		Expected Result		
		Positive		
NEO Iris	Positive	279	% Agreement	98.6%
	Negative	4	Concordance (95% Lower Bound One-Sided CI)	96.8%

Results are for North America Market assays. PPA lower 95% CI did not meet 99% due to 4 false-negative test results.

For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

Capture-R Ready-Screen meets the requirements of the FDA for reagent red blood cells for use in the detection of unexpected antibodies. No US Standard of potency exists for these products.

The expiration date of Capture-R Ready-Screen stripwells is set at 120 days from the date of manufacture which is the earliest date that blood is withdrawn from any donor used in this component.

Bibliography:

1. Boral LI, Henry JB. The type and screen: a safe alternative and supplement in selected surgical procedures. *Transfusion* 1977;17:163.
2. Giblett ER. Blood group alloantibodies: an assessment of laboratory practices. *Transfusion* 1977;17:299.
3. Roualt CL. Appropriate pretransfusion testing, In: *Pretransfusion testing for the '80's*. Washington DC: American Association of Blood Banks, 1980:125.
4. Brecher ME, ed. *Technical manual*. 14th ed. Bethesda, MD: American Association of Blood Banks, 2002.
5. Plapp FV, Sinor LT, Rachel JM et al. A solid phase antibody screen. *Am J Clin Pathol* 1984;82:719.
6. Juji T, Kano K, Milgrom F. Mixed agglutination with platelets. *Int Arch Allergy* 1972;42:474.
7. Beck ML, Rachel JM, Sinor LT et al. Semiautomated solid phase adherence assays for pretransfusion testing. *Med Lab Sci* 1984;41:374.
8. Rolih SD, Eisinger RW, Moheng MC et al. Solid phase adherence assays: alternatives to conventional blood bank tests. *Lab Med* 1985;16:766.
9. Rolih S, Thomas R, Fisher F et al. Antibody detection errors due to acidic or unbuffered saline. *Immunohematology* 1993;9:15.

US License 886 only applies to (Capture-R Ready-Screen Test Wells)

Insert code **346-14**
Rev 7/19



Key:
Underline = Addition or significant change; ▲ = Deletion of text