



REF Cat. No. MAX-ACT

INTENDED USE

The MAX-ACT Activated Clotting Time (ACT) test is intended for use in the performance of the activated clotting time test, a whole blood coagulation assay commonly used to monitor heparin anticoagulation during various medical and surgical procedures. MAX-ACT test tubes can be used in conjunction with the Actalyke instrument, the Actalyke MINI and the Actalyke XL. MAX-ACT test tubes can also be used in conjunction with Hemochron® instruments.

SUMMARY

During extracorporeal circulation (ECC) procedures such as cardiopulmonary bypass (CPB), the patient's blood flows through an extracorporeal circuit. The non-biological surfaces of the bypass circuit are known to have a strong procoagulant effect on the blood. To offset this biological response, anticoagulants are routinely administered. The most commonly used anticoagulant is heparin, and it is usually given in high concentrations during periods of extracorporeal circulation. During procedures involving intense heparinization, the ACT and other endpoint-based coagulation assays are frequently used to monitor heparin effect, heparin concentration, and other coagulation parameters.

Blood clot formation is a complex process. The clotting proteins, or factors, circulate normally in an inactive state as precursors to coagulation. In principle, a series of reactions occurs that in turn acts to catalyze the next reaction, hence the common term "Coagulation Cascade". During the reaction process, these proteins and the resulting fibrin mass itself are unstable and water-soluble. In addition, in the presence of anticoagulants and/or diluted amounts of the coagulation proteins, clotting becomes delayed or prolonged. Eventually, however, fibrin (the foundation of a blood clot) will be formed when fibrinogen, one of the clotting proteins, is cleaved.

History of the ACT

In 1966, Dr. Paul G. Hattersley, a pathologist in California, developed the activated clotting time test.² Dr. Hattersley was seeking a rapid bedside test that was sensitive to coagulation factor deficiencies, especially deficiencies of hemophilic Factors VIII and IX. It is noteworthy that the Hattersley ACT achieved this goal and was indeed very sensitive to such Factor deficiencies (less than 20% Factor activity). The sensitivity of the Hattersley ACT, especially to Factor VIII and IX deficiencies, validated the ACT as a rational, predictable measurement of the (intrinsic) coagulation process. Further test utility included using the ACT for therapeutic management and dosing of Factor VIII concentrates for classic hemophilia.

The Hattersley ACT test principle involved saturating the patient sample with a particulate activator, thereby (in theory) ensuring that all available Factor XII was being converted to Factor XIIa (the activated form of Factor XII). The unique mechanism of the test utilized the intrinsic pathway of coagulation by activating the contact activation system, thus initiating an enzymatic cascade from one factor to another. This reaction was timed "until signs of the first unmistakable clot".^{2,3}

The Becton Dickinson Company (Franklin Lakes, NJ) eventually commercialized the Hattersley ACT test. As described by Dr. Hattersley, the formula included commercially available inert siliceous earth (diatomite-exoskeleton remains of diatomaceous earth, or celite).

ACT utilization during CPB achieved major recognition in the mid to late 1970's. During this time, several ground breaking studies utilizing the Hattersley ACT or a similar derivative were performed. Primarily, these studies addressed target values and heparin and ACT usage during CPB. Different heparinization regimens and target ranges were utilized in these studies with investigators' findings suggesting therapeutic ACT target values ranging from 300 to 600 seconds. Review of this literature included studies by Mattox, Hill, and Bull.

As a result of a thorough study by Bull and colleagues in which several heparinization protocols were analyzed using the ACT¹, a target ACT of 480 seconds evolved as the standard of care for patients undergoing CPB. Among these included a calculation of heparin requirements based on the patient's heparin response and metabolism.

At the same time that Bull, et al were defining ACT target ranges, automation of the original Hattersley ACT had occurred. Ultimately, several different techniques were developed, but all offered an automated endpoint detection, automated temperature management, and automated timing counters. Over the years, numerous publications have been written assessing the strengths, weaknesses, and result differences of these various systems. One interesting point, however, is that all of these ACT systems terminated the testing cycle when a significant clot mass had formed. This clot mass needed to be of sufficient size and strength to displace a magnet or slow a flag motion. This definition of a "clot" is glaringly different from the original ground breaking work on the ACT and its target values. The originator of the method, Dr. Hattersley states, "Five seconds to a minute or more may elapse between the appearance of the first visible clot and the solid coagulation of the entire tube. Erroneously long coagulation times may therefore result from lack of care in observing the first visible clot."² Even in the early stages of development and clinical utilization of the ACT, it was well known that the period between the onset of the clot and the solid clot formation was variable. As a result, any delay in identifying clot onset could lead to an overestimation of the anticoagulant response.

Many automated ACT tests still in use today lack sensitivity to the onset of clotting and instead detect the endpoint as a stable clot. Unfortunately, this detection principle is one of the major contributors to poor ACT test reproducibility. In fact, environmental concerns, reproducibility questions, and a general misunderstanding of the ACT has led to its extinction in the clinical laboratory - in any format.

Biological Improvements: The MAX-ACT Test

For years, it has been assumed that the science behind an ACT test was relatively simple. The premise has been: place an activator in the tube, add the blood sample, and a clot will form. In actuality the formation of the clot is simple, but getting the clot to form within a certain timeframe, location, and strength is not as predictable.

The novel, patented MAX-ACT test represents a new approach to ACT formulation with the goal of standardizing contact activation for enhanced testing reliability. The original handheld ACT described a technique in which the ACT tube was inverted and mixed at least once every 30 seconds throughout the testing period.^{1,3} This documented mixing interval continually "re-exposed" the blood sample to the large amounts of glass of the tube walls. The MAX-ACT uses a new formulation to recapture the original premise of the test by including glass beads as an additional activator thus offering constant blood exposure to glass.

Further, the additional activators speed up the clotting cascade via the simultaneous activation of Factor XII (Hageman Factor). Various commercial preparations of activators such as diatomaceous earth (celite), kaolin, glass-beads, and silica will not initiate Factor XII similarly. In principle, reagent that better activates the clotting cascade (either through quantity or quality of the activator) yields shorter, more reproducible clotting times for all specimens tested throughout the range of the test. This is especially important in CPB where conditions such as hemodilution lower the amount of Factor XII in the blood. Since coagulation is an enzymatic reaction, dependent upon the temperature of the reaction environment, the hypothermic conditions encountered in CPB also come into play.

To overcome the aforementioned variables that affect the blood specimen, it is logical to "oversaturate" and vary the type of Factor XII activation. It is imperative to oversaturate Factor XII at the onset of the reaction to guarantee that the eventual prothrombin to thrombin conversion is maximized, thus reducing extraneous testing variables for enhanced reliability.

Assuming that all of the patient's Factor XII has been activated, the ACT reaction will proceed rapidly with prolongation from the baseline being representative of the degree of heparin anticoagulation. If all of the Factor XII in the patient specimen has not been converted to Factor XIIa, there could be a prolongation in the ACT that may not be heparin related, yet could be (mis)construed as heparin anticoagulation.

Therefore, when developing the MAX-ACT test, an intentional oversaturation of activators was selected, using varied particulate activators to convert all of the patient's Factor XII quickly and reliably. Oversaturating the reaction must be distinguished from "overactivation" of the ACT, which is an erroneous notion, since the patient has a finite amount of coagulation factors (i.e., Factor XII).

Another important aspect of the MAX-ACT is its "cocktail" formulation. Since individuals will respond differently to various activators, most likely due to the multispecies nature of Factor XII⁴, it is beneficial to include multiple activators in each ACT test. This is to ensure that the TOTAL patient population is achieving maximum Factor XII activation via both variety and volume of the activator.

By using the MAX-ACT reagent cocktail, the patient population is in essence being standardized from the perspective of Factor XII activation. In addition, by incorporating a variety of particulate activators in the reagent, any potential patient sensitivity to a single activator will be compensated by the other activators.

PRINCIPLE

The ACT test was first described by Paul Hattersley, M.D., in 1966. Although the test was originally a manual method, automated ACT instruments were later introduced which improved the convenience of the test. Automated test systems like Actalyke feature pre-loaded, disposable test tubes to which a blood sample is added; the test tube is then inserted into the instrument where the tube is rotated and warmed to 37°C (± 0.5°C) until a fibrin clot is mechanically detected. Upon clot detection the test terminates, a buzzer is sounded, and the ACT result is displayed (in seconds) on the instrument LED.

Each Actalyke test tube has a barcode label affixed to it, which is read by the Actalyke instrument to determine the test activator type. [Barcode reader not available on Actalyke MINI models]. The lot number and expiration date of each tube are also identified on the barcode label.

REAGENT

MAX-ACT Tubes

Ingredients: Tubes contain "cocktail" activator containing celite, kaolin, and glass particles.

WARNING: FOR IN-VITRO DIAGNOSTIC USE. CAUTION: Some data suggests that celite is a possible carcinogen. Avoid contact with skin. Used tubes should be disposed of as a biohazardous material.

Recommended Use: Extracorporeal circulation, angioplasty, thoracic and vascular surgery (in the presence or absence of antifibrinolytics).

Storage and Stability: MAX-ACT test tubes can be stored at room temperature (15-30°C). They must not be used past the marked expiration date.

INSTRUMENTS

The MAX-ACT test tubes should be used with the Actalyke, Actalyke Mini, Actalyke XL or Hemochron® instruments. Refer to the appropriate Operator's Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Use a 2.0 cc syringe to obtain the blood specimen as follows:

Extracorporeal line: Using a two-syringe technique, flush the extracorporeal blood access line by withdrawing 2.0 cc of blood into a syringe and discarding it. Then use a second syringe to obtain a 0.5 cc sample for testing.

In-dwelling line: Discontinue fluids drip, if necessary. Using a two-syringe technique, withdraw 2.0 cc of blood into a syringe and discard it. Then use a second syringe to obtain a 0.5 cc sample for testing.

Venipuncture: Using a two-syringe technique, withdraw 2.0 cc of blood into a syringe and discard it. Then use a second syringe to obtain a 0.5 cc sample for testing.

PROCEDURE

Materials Provided:

MAX-ACT Test Tubes (50/box)

Materials Required:

	Cat. No.
Actalyke XL	5770
Actalyke XL Barcode Reader & Cable	5758
Actalyke MINI	5752
Actalyke MINI with printer	5750
Actalyke Clotting Time Analyzer	
Single Well	A1
Single Well with printer	A1P
Dual well	A2
Dual well with printer	A2P
Actalyke Thermometer	5757
Actalyke QC Kit (Level I & II)	AQC-LP
Actalyke QC Kit (Level I & III)	AQC-HP
Electronic Clotting Tube	XL-ECT
Syringes for sample collection	

STEP-BY-STEP METHOD

NOTE: Refer to the appropriate Operator's Manual for detailed instrument instructions. Refer to the SAMPLE COLLECTION section for detailed collection instructions.

1. Open the flip-top of the MAX-ACT test tube.
2. Dispense 0.5 cc of blood into the MAX-ACT tube, filling to the line indicated.
3. Perform the next steps quickly and in order depending on the type of instrument settings used.

- ◇ Press the Start button at the same time that the blood is added to the tube.
- ◇ Close the flip-top. A gentle side-to-side shake should be used. **DO NOT AGITATE THE TUBE FROM END TO END.**
- ◇ Insert the MAX-ACT tube into the instrument test well.
- ◇ Rotate the tube 4-5 times; the green detector light will illuminate or "Tube In" will display

NOTE: This mixing procedure eliminates protein loss to the tube walls and assists in standardizing the mixing procedure from operator to operator.

4. Upon clot detection, the buzzer will sound and the ACT test result will be displayed in seconds. (Results can be printed if using Actalyke Models A1P, A2P Actalyke XL, or Actalyke MINI with printer.)

Quality Control

Routine quality control testing and tracking should be part of a comprehensive quality assurance program. According to the CLIA '88 regulations, the frequency of quality control testing is mandated. Since the ACT is categorized as moderately complex, ACT users must (1) perform two levels of quality control during each shift in which the test system is used clinically, and (2) subscribe to a Proficiency Testing program. Additionally, CLIA (42 CFR 493) requires that biological controls be used weekly to verify system function. To help users comply with these regulations, Actalyke QC Kits (Cat. No. AQC-LP, AQC-HP) (or other commercial coagulation products can also be used) are available to make routine QC convenient and affordable. ACT Proficiency Testing Programs are available through American Proficiency Institute and the College of American Pathologists.

Each box of Actalyke ACT tubes contains 50 (single box) unitized reaction tubes from a single manufactured lot. A sampling from a box of tubes from each lot number of each shipment should be validated once, initially upon arrival. This can be accomplished using the appropriate Actalyke QC Kit. Acceptable performance ranges for the various Actalyke coagulation assays are included in each kit. After each lot number of Actalyke Activated Clotting Time tubes has been verified with the QC kit, that lot of tubes should be labeled as "VERIFIED", along with the date of verification and the initials of the operator. This lot is now "IN CONTROL" and will not require any further investigation until a new shipment of the same lot or a new lot of tubes is received. Further testing is required if the tubes are not stored to manufacturers recommendations (please refer to proper storage details), or if a shift in clinical results is experienced.

Quality Control results that fall outside the established expected values should be repeated. If the problem persists, the source should be investigated and corrected prior to continued use of the test system.

Operating Cautions and Limitations

1. Do not use Actalyke ACT test tubes that are past the expiration date marked on the tube barcode label and the corresponding test tube box.
2. All guidelines pertaining to the handling of fresh whole human blood should be adhered to when handling Actalyke test tubes and instruments.
3. Specimen contamination and inappropriate handling technique can affect ACT results.
4. Hemodilution, hypothermia, pharmacologic compounds, and various coagulopathies may affect ACT results. Test results should be interpreted with respect to the patient's condition and the clinical circumstances. Those results which do not agree with expected values should be repeated and further evaluated by other diagnostic methods, if indicated.

REFERENCE RANGES

MAX-ACT test tubes were run on normal healthy patients using multiple Actalyke (Models XL, A2P and MINI) and Hemochron Instruments (Model 8000). Quality control tests were performed on each instrument prior to testing of Actalyke tubes for this study. The results were as follows:

	N	Mean	2SD	Reference Range
Actalyke XL	66	118	17	100-136 sec.
Actalyke MINI	49	115	18	97-133 sec.
Actalyke A2P	49	117	22	98-136 sec.
Hemochron		112	17	95-129 sec.

Mean data from each patient was used to establish the mean \pm 2SD normal range. Each laboratory should perform its own normal range study.

PERFORMANCE CHARACTERISTICS

Clinical Data Performance

Studies were also conducted clinically at numerous institutions. A total of 330 paired blood samples were collected from patients (including adult bypass, pediatric bypass, and cardiac catheterization) before, during, and following heparinization.

Using a reference celite-based ACT test (FTCA510/C-ACT) in CPB patients, the data yielded a correlation coefficient of $r^2 = 0.82$ and $r^2 = 0.89$ when samples from the reference group were omitted which were outside the published linear range (0-600 seconds) for the reference tube.

Results obtained using a reference kaolin-based ACT test (ACTII/K-ACT/FTKACT) were compared to those obtained using MAX-ACT test tubes, and the data yielded a correlation coefficient of $r^2 = 0.89$.

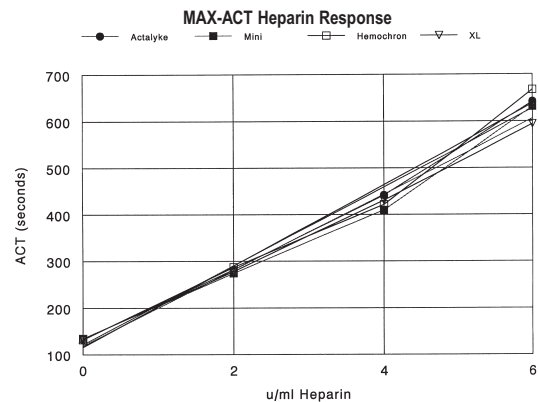
Correlation Data

The Actalyke XL and the MINI were compared to the Actalyke using MAX-ACT tubes. The data was as follows:

MINI	n=166	Y=0.995X - 4	r=0.989
XL	n=104	Y=0.983X + 2.7	r=0.985

Heparin Sensitivity

Heparin response was determined with multiple heparin concentrations added to the blood of normal donors. Curves were generated using the mean of the pooled data from 5 donors (r values are >0.99) yielding the following results.



Heparin Linearity

Linearity studies were done according to NCCLS EP-6 guidelines. The MAX-ACT demonstrated an upper linearity limit of 6 units of heparin per milliliter of patient blood.

Precision Data

All precision studies were done according to NCCLS EP-5 guidelines.

Actalyke MINI

The precision of the MAX-ACT tubes with the Actalyke Mini ACT system was evaluated by performing multiple replicates on twenty separate days with heparinized and non-heparinized Actalyke Whole Blood Control samples, levels I and III.

Level	I	III
mean	125.5	401.9
SD	3.57	23.54
%CV	2.8	5.9

Actalyke XL

The precision of the MAX-ACT tubes with the Actalyke XL ACT system was evaluated by performing multiple replicates on twenty separate days with heparinized and non-heparinized Actalyke Whole Blood Control samples, levels I and III.

Level	I	III
mean	126.3	402.2
SD	4.5	25.4
%CV	3.5	6.3

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